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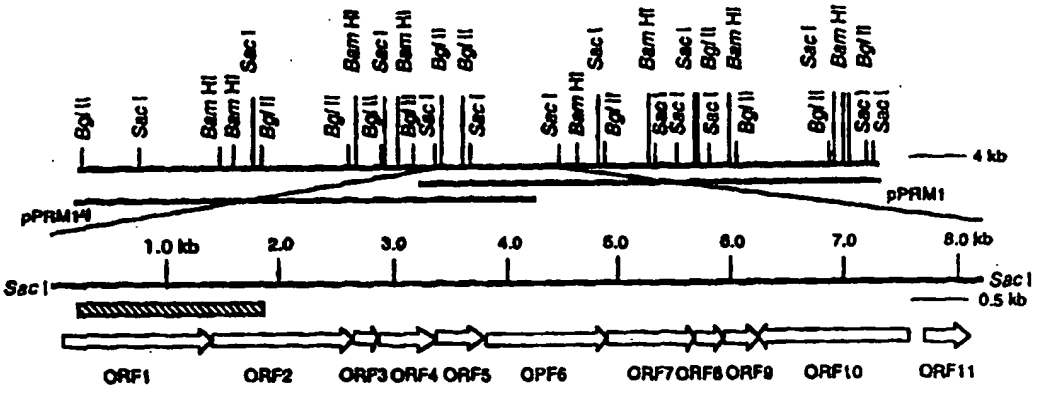


(57) Abstract

The present inventioo provides, *inter alia*, nucleic acids and corresponding amino acid sequences of several *Actinomadura* polyketide synthase genes that are useful, for exemple, in preparing pradimicin and analogs thereof.

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# POLYKETIDE SYNTHASES FOR PRADIMICIN BIOSYNTHESIS AND DNA SEQUENCES ENCODING SAME

The present invention relates, *inter alia*, to purified nucleic acids encoding polyketide synthase genes for pradimicin biosynthesis, and purified polypeptides having polyketide synthase activity. Polyketide metabolites are natural products made by microorganisms and plants from simple fatty acids. Many polyketides are used as human and animal pharmaceuticals such as antibiotics, chemotherapeutics and growth promoting agents, as well as flavoring agents and pigments.

Biosynthesis of polyketides is believed to occur by a series of condensations of carbon units in a manner similar to that of long chain fatty acids which are formed by fatty acid synthase. The fatty acids are formed by a process in which a chain starter, usually a 2-carbon acetate residue, which is joined by condensation to a chain extender unit, such as malonate, to form an even-numbered chain. The resulting β-keto group is then processed, by β-ketoacyl reduction, dehydration and enoyl reduction. The cycle then begins again with the condensation of a new extender unit. A typical fatty acid synthase is a multivalent system involving eight functional units, acetyl, malonyl and palmityl transferases, acyl carrier protein, ketoacyl synthase, ketoacyl reductase, dehydratase and enoyl reductase. The organization of these units varies in different organisms. See, for example, *EMBO J.* 8:2717-2725 (1989).

The fatty acid synthesis process differs from polyketide synthesis since most polyketides contain structural complexities due to the use of different starter and extender units, such as acetate, propionate and butyrate. The polyketide synthesis is further complicated by variations in the extent of processing of the β-carbon (β-ketoreduction, dehydration, enoylreduction) as well as the introduction of chiral carbons. See, for example, *Science* 252:675-679 (1991).

The tetracenomycin C polyketide synthase genes (*tmcI*) from *Streptomyces glaucescens*, for example, have been sequenced, and the

sequence data revealed three complete open reading frames. An analysis of the sequence data resulted in a conclusion that polyketide synthesis in *S.glaucescens* involves a multienzyme complex consisting of at least five types of enzymes. These enzymes, which are homologous to counterparts involved in fatty acid synthesis, are presumably involved in the assembly of the tetracenomycin C decaketide.

Additionally, for example, the structure and function of the granaticin-producing polyketide synthase gene cluster of *Streptomyces violaceoruber* has also been studied. This gene cluster has six open reading frames, thereby indicating that the granaticin-producing polyketide synthesis likely consists of at least six separate enzymes involved in carbon chain assembly. See *EMBO J.* 8:2717-2725 (1989). Further, *Streptomyces* polyketide synthase gene clusters involved in the biosynthesis of actinorhodin and the *whiE* spore pigment have also been described. See *J. Biol. Chem.* 267:19278-19290 (1992) and *Gene* 130:107-116 (1993).

The molecular organization of the polyketide biosynthesis genes of *Saccharopolyspora erythraea*, which govern synthesis of the polyketide portion of the macrolide antibiotic erythromycin, is similarly complex. The genes are organized in six repeated units that encode fatty acid synthase-like activities. Two repeated units are contained in a single open reading frame. It is believed that each repeated unit encodes a functional synthase unit and each synthase unit participates in one of six fatty acid synthase-like elongation steps required for the formation of the polyketide. See *EMBO J.* 8:2727-2736 (1989).

Based on the above data, a model has been proposed in which polyketide genes have repeated units designated modules, and the corresponding proteins are called synthase units, wherein each synthase unit is responsible for one of the fatty acid synthase-like cycles required for completing the polyketide. Thus, each synthase unit carries the

elements required for the condensation process, for selecting the particular extender unit to be incorporated, and for the extent of processing that the β-carbon will undergo. After completion of the cycle, the nascent polyketide is transferred from the acyl carrier protein (ACP) it occupies to the β-ketoacyl ACP synthase of the next synthase unit utilized, where the appropriate extender unit and processing level are introduced. This process is repeated, using a new synthase unit for each elongation cycle, until the programmed length has been reached. According to this model, formation of complex polyketides requires the participation of a different synthase unit for each cycle, thereby ensuring that the correct molecular structure is produced. See, for example, *Annu. Rev. Microbiol.* 47:875-912 (1993).

An actinomycete, namely, *Actinomadura*, certain strains of which were previously isolated from soil samples collected in the Fiji Islands and in India, was found to produce a complex of antibiotics designated pradimicin. See, for example, *J. Antibiot.* 43:755-762 (1990). Pradimicin A, as shown in Figure 1, has a unique dihydro-benzo[*a*]naphthacenequinone aglycon substituted with D-alanine and two sugars, and is a potent antifungal antibiotic produced, for example, by *Actinomadura hibisca* and *Actinomadura verrucosospora* subsp. *neohibisca*. See, for example, *J. Antibiot.* 43:755-762 (1990) and *J. Antibiot.* 46:387-397 (1993). Pradimicin is an antibiotic useful for multiple purposes, particularly for use as a pharmaceutical. For example, pradimicin has been shown to have activity against system fungal infections caused by *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*. Further, pradimicin is active *in vitro* against a wide variety of fungi and yeasts, some Gram-positive bacteria, and viruses. *J. Org. Chem.* 54:2536-2539 (1989). Purified polypeptides having polyketide synthase activity and purified nucleic acids encoding such polypeptides are therefore desirable, for example, to provide pharmaceutically useful products.

## SUMMARY OF THE INVENTION

Until now, the sequences encoding polyketide synthase genes in *Actinomadura* had not been identified. These sequences are provided in the present invention.

One preferred embodiment of the present invention is a substantially pure nucleic acid comprising a nucleic acid sharing at least about 75% nucleic acid identity with an open reading frame (ORF) of an *Actinomadura* polyketide synthase gene, and more preferably, at least about 80% identity, and most preferably, at least about 90% identity. In certain preferred embodiments, the nucleic acid comprises a nucleic acid selected from the group consisting of SEQ ID NO:1-12. A further preferred embodiment is a substantially pure nucleic acid comprising a nucleic acid encoding an *Actinomadura* polyketide synthase gene sharing at least about 75% amino acid identity, and more preferably, at least about 80% identity, and most preferably, at least about 90% identity with a polypeptide encoded by a nucleic acid selected from the group consisting of SEQ ID NO:1-12.

In certain preferred embodiments, the substantially pure nucleic acid comprises a nucleic acid encoding a polypeptide differing from an *Actinomadura* polyketide synthase gene by no more than about 20 amino acid substitutions, and more preferably, no more than about 10 amino acid substitutions. Preferably, the substitutions cause a conservative substitution in the amino acid sequence of the encoded polyketide synthase. The nucleic acids of the invention also include nucleic acid analogs.

Further, the present invention provides a substantially pure nucleic acid comprising a nucleic acid encoding a polypeptide sharing at least about 75% amino acid identity with a polyketide synthase for biosynthesis of a benzo(*a*)naphthacenequinone. Preferably, the nucleic acid encodes a polypeptide sharing at least about 80%, and more preferably, at least about 90% amino acid identity with a polyketide

synthase for biosynthesis of a benzo(*a*)naphthacenequinone. In preferred embodiments, the polyketide synthase is an *Actinomadura* polyketide synthase, and the polyketide is preferably a dihydrobenzo(*a*)naphthacenequinone aglycon, and preferably pradimicin, such as Pradimicin A, B, C, D, E, FA-1, FA-2, FL, FS, H, 11-*O*-L-xylosylpradimicin H, L, S, T1, T2 or BMS181184.

Yet another embodiment of the invention is a substantially pure nucleic acid comprising a nucleic acid that hybridizes, under stringent conditions, to a nucleic acid comprising a nucleic acid encoding a polypeptide sharing at least about 75% amino acid identity with an actinomadura polyketide synthase. More preferably, the nucleic acid hybridizes to a nucleic acid comprising a nucleic acid encoding a polypeptide sharing at least about 80% amino acid identity with an *Actinomadura* polyketide synthase, and even more preferably, encoding a polypeptide sharing at least about 90% amino acid identity with an *Actinomadura* polyketide synthase. Most preferably, the nucleic acid hybridizes with a nucleic acid comprising a nucleic acid selected from the group consisting of SEQ ID NO:1-12. Such a hybridizing nucleic acid can be used, for example, to screen for organisms that produce pradimicin.

The invention additionally includes vectors capable of reproducing in a eukaryotic or prokaryotic cell having a nucleic acid described above as well as transformed eukaryotic or prokaryotic cells having such nucleic acid.

Thus, another preferred embodiment is a transformed eukaryotic or prokaryotic cell comprising a nucleic acid encoding a polypeptide sharing at least about 70% amino acid identity with an *Actinomadura* polyketide synthase gene, and more preferably, at least about 80% identity, and most preferably, at least about 90% identity. Most preferably, the nucleic acid sequence comprises a nucleic acid selected from the group consisting of SEQ ID NO:1-12. Preferably, the

transformed cell expresses one of the *Actinomadura* polyketide synthase genes described herein.

Yet another preferred embodiment is a vector capable of reproducing in a eukaryotic or prokaryotic cell comprising a nucleic acid encoding a polypeptide sharing at least about 70% nucleic acid identity with an *Actinomadura* polyketide synthase gene, and more preferably, at least about 80% identity, and most preferably, at least about 90% identity. Preferably, the nucleic acid comprises a nucleic acid selected from the group consisting of SEQ ID NO:1-12. Preferably, the inventive vector expresses, intracellularly or extracellularly, one of the *Actinomadura* polyketide synthases described herein.

Another embodiment of the present invention provides a substantially pure polypeptide comprising an amino acid sequence sharing at least about 75% amino acid identity with an *Actinomadura* polyketide synthase, and more preferably, at least about 80% identity, and most preferably, at least about 90% identity. Preferably, the polypeptide shares at least about 75% amino acid identity with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:13-15.

Yet another preferred embodiment is a method of preparing pradimicin or a pradimicin analog thereof, comprising transforming a eukaryotic or prokaryotic cell with an expression vector for expressing intracellularly or extracellularly a nucleic acid comprising a nucleic acid encoding a polypeptide sharing at least about 70% amino acid identity with an *Actinomadura* polyketide synthase, growing the transformed cell in culture, and isolating the pradimicin or analog thereof from the transformed cell or the culture medium. Preferably, the polypeptide shares at least about 80% amino acid identity with an *Actinomadura* polyketide synthase, and more preferably, the polypeptide shares at least about 90% amino acid identity with an *Actinomadura* polyketide synthase. Most prefereably, the expression vector comprises a nucleic

acid encoding all polyketide synthase genes necessary for synthesis of pradimicin, such as SEQ ID NO:1.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the chemical structure of two types of pradimicin, pradimicin A and pradimicin S.

Figure 2 shows conserved amino acid sequences in β-ketosynthases and acyl transferases for granaticin, tetracenomycin and actinorhodin. These conserved sequences were used to create two probes for cloning the polyketide synthase genes in *Actinomadura*.

Figure 3 shows a restriction map of *Actinomadura* polyketide synthase genes, ORFs 1-11.

Figure 4 provides an alignment of the *Actinomadura* ORF1 gene product ("A") (SEQ ID NO:13) with a *Streptomyces* polyketide synthase gene product for tetracenomycin biosynthesis ("B").

Figure 5 provides an alignment of the *Actinomadura* ORF2 gene product ("A") (SEQ ID NO:14) with a *Streptomyces* polyketide synthase gene product for actinorhodin biosynthesis ("B").

## DETAILED DESCRIPTION

The present invention provides, *inter alia*, nucleic acids and corresponding amino acid sequences of *Actinomadura* polyketide synthase genes. The polyketide synthases are responsible for the biosynthesis of pradimicin, such as zwitterionic pradimicins A, B and C, which are produced, for example, by *Actinomadura hibisca*, and pradimicin S, which is produced, for example, by *Actinomadura spinosa*.

See Figure 1, which provides the chemical structures of pradimicins A and S. See also *J. Antibiot.* 43:755-762 (1990). Pradimicin is useful, for example, as an antibiotic, including use as an anti-fungal and an anti-viral agent. For example, pradimicin has been shown to have activity against system fungal infections caused by *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*. Further, pradimicin is active *in vitro* against a wide variety of fungi and yeasts, some Gram-positive bacteria, and viruses. *J. Org. Chem.* 54:2536-2539 (1989). For instance, pradimicin is believed to be active against HIV. See, for example, *J. Antibiot.* 41:1708 (1988) and *Virology* 176:467 (1990).

Techniques used in the prior art were not applicable for cloning pradimicin A biosynthetic genes from *Actinomadura hibisca*. Specifically, many antibiotic biosynthetic genes including self-defense genes in actinomycetes are clustered in a genomic region. The close linkage between antibiotic biosynthetic genes and self-defense genes has provided a useful tool for cloning of antibiotic biosynthetic genes, since transformants carrying antibiotic resistance determinants can be selected. However, this technique could not be applied to the cloning of the pradimicin A biosynthetic gene cluster because pradimicin A had not been shown to have significant antibacterial activity. Therefore, the polyketide synthase genes for pradimicin A biosynthesis were cloned from *Actinomadura hibisca* using oligonucleotide probes based on the conserved amino acid sequences of other polyketide synthase genes, followed by cloning of the flanking region of pradimicin A polyketide synthase genes. Specifically, certain amino acid sequences of ß-keto synthase, acyl transferase and acyl carrier protein of polyketide synthases are strongly conserved in *Streptomyces* strains producing polyketide antibiotics. See *Annu. Rev. Microbiol.* 47:875-912 (1993) and *J. Biol. Chem.* 267:19278-19290 (1992). Based on these sequences, two oligonucleotide probes were synthesized, as shown in

Figure 2. See also Example 1, which provides experimental details of the cloning of the pradimicin A polyketide synthase genes.

After screening with an *Actinomadura hibisca* library, an 8.2 kb *Sac* I fragment was identified, which hybridized with these oligonucleotide probes. By DNA sequencing of the 8.2 kb *Sac* I fragment (SEQ ID NO:1), eleven open reading frames (ORFs) were identified. All of ORFs except for ORF10 are believed to be translated in the same direction. Referring to SEQ ID NO:1, ORF1 spans from position 72 (beginning with GTG) to position 1347 (ending with TGA); ORF2 spans from 1346 (GTG) to 2567 (TGA); ORF3 spans from 2594 (ATG) to 2855 (TGA); ORF4 spans from 2854 (ATG) to 3313 (TGA); ORF5 spans from 3312 (GTG) to 3771 (TGA); ORF6 spans from 3794 (ATG) to 4817 (TGA); ORF7 spans from 4857 (ATG) to 5595 (TGA); ORF8 spans from 5594 (GTG) to 5933 (TGA); ORF9 spans from 5932 (GTG) to 6241 (TAA); ORF10 spans, in reverse direction, from 7534 (ATG) to 6301 (TAG) and ORF11 spans from 7668 (ATG) to 8010 (TGA).

Each of the deduced ORFs has a significant similarity to a protein responsible for polyketide biosynthesis or spore color formation in other organisms. ORF1, ORF2 and ORF3 have particularly strong similarities (50% - 70% amino acid identity) with polyketide synthases for actinorhodin biosynthesis. See, for example, Figure 4, which provides an alignment of the ORF1 gene product with a *Streptomyces* polyketide synthase gene product for tetracenomycin biosynthesis, and Figure 5, which provides an alignment of the ORF2 gene product with a *Streptomyces* polyketide synthase gene product for actinorhodin biosynthesis. See also Table 1 below.

**Table 1**

| ORFs | Number of amino acids | Molecular weight | Translational coupling | Homologous proteins |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| ORF1 | 426 | 44,440 | Unknown | Hypothetical protein 4 of Sac. *hirsuta* (73% identity among 413 amino acids)[1]<br>*tcm* Ia gene of S. *glaucescens* (73%/412)[2]<br>*gra* I gene of S. *violaceruber* (71%/413)[3]<br>*act* I ORF1 of S. *coelicolor* (69%/415)[4] |
| ORF2 | 408 | 41,610 | ORF1/ORF2 | *act* I ORF2 of S. *coelicolor* (57%/397)[4]<br>*tcm* Id gene of S. *glaucescens* (54%/403)[2]<br>Beta-ketoacyl synthase chain 2 of S. *cinnamonensis* (50%/397)[5] |
| ORF3 | 88 | 9,688 | — | Hypothetical protein 6 of Sac. *hirsuta* (51%/78)[1]<br>Granaticin-producing PKS acyl carrier protein of S. *violaceruber* (53%/75)[3]<br>Actinorhodin-producing PKS acyl carrier protein of S. *coelicolor* (51%/75)[4] |
| ORF4 | 154 | 17,694 | ORF3/ORF4 | Hypothetical protein 7 of S. *coelicolor* (58%/149)[6]<br>PKS cyclase *cur*F of S. *cyaneus* (61%/142)[7]<br>*tcm*N protein of S. *glaucescens* (52%/149)[8] |
| ORF5 | 154 | 15,784 | ORF4/ORF5 | Hypothetical protein 6 of *Mixococcus xanthus* (46%/39)[9]<br>Histidine protein kinase *div*J of *Caulobacter crescentus* (26%/102)[10]<br>Multicatalytic endopeptidase complex chain Y7 of *Sac. cerevisiae* (23%/105)[11] |
| ORF6 | 342 | 37,004 | — | *tcm*N protein of S. *glaucescens* (47%/330)[8]<br>Carminomycin 4-O-methyltransferase of S. *peucetius* (30%/317)[12]<br>O-demethylpuromycin O-methyltransferase of S. *anulatus* (33%/334)[13] |
| ORF7 | 247 | 25,583 | — | 3-ketoacyl-ACP reductase *fab* G of E. *coli* (38%/244)[14]<br>Granaticin-producing PKS chain 5 of S. *violaceruber* (30%/251)[3]<br>Granaticin-producing PKS chain 6 of S. *violaceruber* (35%/252)[3] |
| ORF8 | 114 | 12,986 | ORF7/ORF8 | Hypothetical protein 1 of S. *coelicolor* (24%/80)[6] |

| | | | | |
|---|---|---|---|---|
| ORF9 | 104 | 11,279 | ORF8/ORF9 | Hypothetical protein 1 of S. *coelicolor* (24%/91)[2]<br>Hypothetical protein 6 of *Sac. hirsuta* (27%/48)[11]<br>Hypothetical 41.2 KD protein of *S. halstedii* (24%/91)[15] |
| ORF10 | 412 | 44,857 | — | Cytochrome P450 105B1 of S. *griseolus* (40%/404)[16]<br>Cytochrome P450 P450CVIIB1 of *Sac. erythraea* (38%/405)[17]<br>Cytochrome P450 105C1 of *Streptomyces sp.* (41%/323)[18] |
| ORF11 | 115 | 13,036 | — | Hypothetical protein 7 of S. *coelicolor* (51% 107)[5]<br>*cur*G protein of S. *cyaneus* (45%/106)[7]<br>*tcm*I protein of S. *glaucescens* (35%/105)[19] |

1) *Mol. Gen. Genet.* 240:146-150 (1993).

2) *EMBO J.* 8:2727-2736 (1989).

3) *EMBO J.* 8:2717-2725 (1989).

4) *J. Biol. Chem.* 267:19278-19290 (1992).

5) *Mol. Gen. Genet.* 234:254-264 (1992).

6) *Mol. Microbiol.* 4:1679-1691 (1990).

7) *Gene* 117:131-136 (1992).

8) *J. Bacteriol.* 174:1810-1820 (1992).

9) EMBL data library no. S32173.

10) *Proc. Natl. Acad. Sci.* 89:10297-10301 (1992).

11) *Mol. Cell. Biol.* 11:344-353 (1991).

12) *J. Bacteriol.* 175:3900-3904 (1993).

13) *Gene* 109:55-61 (1991).

14) *J. Biol. Chem.* 267:5751-5754 (1992).

15) *Gene* 130:107-116 (1993).

16) *J. Bacteriol.* 173:3335-3345 (1990).

17) *J. Bacteriol.* 174:725-735 (1992).

18) *J. Bacteriol.* 172:3644-3653 (1990).

[19] EMBL data library no. S27691.

DNA regions homologous to the *Actinomadura* polyketide synthase genes were specifically found in all of pradimicin producers examined, but not in pradimicin non-producers in genomic Southern hybridization, thereby providing evidence that the genes cloned encode polyketide synthases for pradimicin biosynthesis.

Thus, the present invention provides, *inter alia*, nucleic acids encoding *Actinomadura* polyketide synthase genes and polypeptides and analogs thereof, including nucleic acids that bind to an *Actinomadura* polyketide synthase gene. The nucleic acids can be used, for example, to screen for organisms that produce pradimicin or that have homologous polyketide synthase gene sequences. Further, the nucleic acids can be used, for instance, to synthesize polyketide synthases, which can in turn be used, for example, to produce pradimicin.

The *Actinomadura* species include but are not limited to *Actinomadura hibisca*, *Actinomadura verrucosospora*, and particularly subsp. *neohibisca*, *Actinomadura libanotica*, *Actinomadura echinospora*, *Actinomadura chengduensis*, *Actinomadura kijaniata*, *Actinomadura atramentaria*, *Actinomadura citrea*, *Actinomadura cremea*, *Actinomadura fulvescens*, *Actinomadura viridis*, *Actinomadura roseoviolacea*, *Actinomadura verrucosopora*, *Actinomadura madurae*, *Actinomadura pelletieri* and, for example, other soil isolates.

**1. Nucleic Acids**

The present invention provides, *inter alia*, nucleic acids. The nucleic acid embodiments of the invention are preferably deoxyribonucleic acids (DNAs), both single- and double-stranded, and most preferably double-stranded deoxyribonucleic acids. However, they can also be ribonucleic acids (RNAs), as well as hybrid RNA:DNA double-stranded molecules.

Nucleic acids encoding an *Actinomadura* polyketide synthase gene include all *Actinomadura* polyketide synthase gene-encoding nucleic acids, whether native or synthetic, RNA, DNA, or cDNA, that encode an *Actinomadura* polyketide synthase gene, or the complementary strand thereof, including but not limited to nucleic acid found in an *Actinomadura* polyketide synthase gene-expressing organism. For recombinant expression purposes, codon usage preferences for the organism in which such a nucleic acid is to be expressed are advantageously considered in designing a synthetic polyketide synthase-encoding nucleic acid.

Further, the present invention provides a substantially pure nucleic acid comprising a nucleic acid encoding a polypeptide sharing at least about 75% amino acid identity with a polyketide synthase for biosynthesis of a benzo(*a*)naphthacenequinone. Preferably, the nucleic acid encodes a polypeptide sharing at least about 80%, and more preferably, at least about 90% amino acid identity with a polyketide synthase for biosynthesis of a benzo(*a*)naphthacenequinone. In preferred embodiments, the
polyketide synthase is an *Actinomadura* polyketide synthase, and the polyketide is preferably a dihydrobenzo(*a*)naphthacenequinone aglycon, and preferably pradimicin, such as Pradimicin A, B, C, D, E, FA-1, FA-2, FL, FS, H, 11-*O*-L-xylosylpradimicin H, L, S, T1, T2 or BMS181184. For a description of the foregoing pradimicins, see, for example, *J. Antibiot.* 41:1701 (1988), *J. Org. Chem.* 54:2536 (1989), *J. Antibiot.* 43:771 (1990), *J. Antibiot.* 43:1223 (1990), *J. Antibiot.* 46:265 (1993), *J. Antibiot.* 46:398 (1993), *J. Antibiot.* 46:406 (1993), *J. Antibiot.* 46:598 (1993), and *J. Antibiot.* 46:1589 (1993).

In addition to nucleic acids encoding an *Actinomadura* polyketide synthase gene, the present invention includes nucleic acids encoding polypeptides that are homologous to or share a percentage amino acid identity with *Actinomadura* polyketide synthases.

Numerous methods for determining percent homology are known in the art. One preferred method is to use version 6.0 of the GAP computer program for making sequence comparisons. The program is available from the University of Wisconsin Genetics Computer Group and utilizes the alignment method of Needleman and Wunsch, *J. Mol. Biol.* 48, 443, 1970, as revised by Smith and Waterman *Adv. Appl. Math.* 2, 482, 1981.

Numerous methods for determining percent identity are also known in the art, such as use of the FASTA computer program, which is also available from the University of Wisconsin. Preferably, the program used to determine percent identity is the DNASIS program, which is available from Hitachi Corp. (Tokyo, Japan).

To construct non-naturally occurring *Actinomadura* polyketide synthase gene-encoding nucleic acids, the native sequences can be used as a starting point and modified to suit particular needs. The nucleic acids of the invention include, for example, the nucleic acids of SEQ ID NO:1-12.

The invention is also directed to a nucleic acid encoding a segment of an *Actinomadura* polyketide synthase gene. Preferably, the encoded polypeptide will be effective to perform its function, such as an enzymatic function, that is performed by the full-size polyketide synthase.

For identifying the active domain or domains of *Actinomadura* polyketide synthase genes, one approach is to take an *Actinomadura* polyketide synthase gene cDNA and create deletional mutants lacking segments at either the 5' or the 3' end by, for instance, partial digestion with S1 nuclease, Bal 31 or Mung Bean nuclease (the latter approach described in literature available from Stratagene, San Diego, CA, in connection with a commercial deletion cloning kit). Alternatively, the deletion mutants are constructed by subcloning restriction fragments of an *Actinomadura* polyketide synthase gene cDNA. The deletional

constructs are cloned into expression vectors and tested for their polyketide synthase activity.

These structural genes can be altered by mutagenesis methods such as that described by Adelman et al., *DNA*, 2: 183 (1983) or through the use of synthetic nucleic acid strands. The products of mutant genes can be tested for polyketide synthase activity.

The nucleic acid sequences can be further mutated, for example, to incorporate useful restriction sites. See Maniatis et al. *Molecular Cloning, a Laboratory Manual* (Cold Spring Harbor Press, 1989). Such restriction sites can be used to create "cassettes," or regions of nucleic acid sequence that are facilely substituted using restriction enzymes and ligation reactions. The cassettes can be used to substitute synthetic sequences encoding mutated *Actinomadura* polyketide synthase amino acid sequences.

*Actinomadura* polyketide synthase gene-encoding sequences can be, for instance, substantially or fully synthetic. See, for example, Goeddel et al., *Proc. Natl. Acad. Sci. USA*, 76, 106-110 (1979). For recombinant expression purposes, codon usage preferences for the organism in which such a nucleic acid is to be expressed are advantageously considered in designing a synthetic *Actinomadura* polyketide synthase gene-encoding nucleic acid. Since the nucleic acid code is degenerate, numerous nucleic acid sequences can be used to create the same amino acid sequence.

Further, with an altered amino acid sequence, numerous methods are known to delete sequences from or mutate nucleic acid sequences that encode a polypeptide and to confirm the function of the polypeptides encoded by these deleted or mutated sequences. Accordingly, the invention also relates to a mutated or deleted version of an *Actinomadura* polyketide synthase nucleic acid that encodes a polypeptide that preferably retains polyketide synthase activity.

Conservative mutations are preferred. Such conservative mutations include mutations that switch one amino acid for another within one of the following groups:

1. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro and Gly;
2. Polar, negatively charged residues and their amides: Asp, Asn, Glu and Gln;
3. Polar, positively charged residues: His, Arg and Lys;
4. Large aliphatic, nonpolar residues: Met, Leu, Ile, Val and Cys; and
5. Aromatic residues: Phe, Tyr and Trp.

A preferred listing of conservative substitutions is the following:

| Original Residue | Substitution |
|---|---|
| Ala | Gly, Ser |
| Arg | Lys |
| Asn | Gln, His |
| Asp | Glu |
| Cys | Ser |
| Gln | Asn |
| Glu | Asp |
| Gly | Ala, Pro |
| His | Asn, Gln |
| Ile | Leu, Val |

| | |
|---|---|
| Leu | Ile, Val |
| Lys | Arg, Gln, Glu |
| Met | Leu, Tyr, Ile |
| Phe | Met, Leu, Tyr |
| Ser | Thr |
| Thr | Ser |
| Trp | Tyr |
| Tyr | Trp, Phe |
| Val | Ile, Leu |

The types of substitutions selected may be based on the analysis of the frequencies of amino acid substitutions between homologous proteins of different species developed by Schulz et al., *Principles of Protein Structure*, (Springer-Verlag, 1978), pp. 14-16, on the analyses of structure-forming potentials developed by Chou and Fasman, *Biochemistry* 13: 211 (1974) or other such methods reviewed by Schulz et al, *Principles in Protein Structure*, (Springer-Verlag, 1978), pp. 108-130, and on the analysis of hydrophobicity patterns in proteins developed by Kyte and Doolittle, *J. Mol. Biol.* 157: 105-132 (1982).

**2. Polypeptides**

In addition to analogs of nucleic acid sequences, the present invention includes analogs of *Actinomadura* polyketide synthases that preferably retain polyketide synthase activity. Preferably, the analogs will share at least about 75% amino acid identity, more preferably, at least about 80% identity, even more preferably, at least about 85%

identity, even more preferably at least about 90% identity, and most preferably at least about 95% identity to an *Actinomadura* polyketide synthase, such as the polypeptide of SEQ ID NO:13, SEQ ID NO:14 or SEQ ID NO:15.

**3. Methods of Synthesizing Polypeptides**

In one embodiment, the polypeptides of the invention are made as follows, using a gene fusion. For example, fusion to maltose-binding protein ("MBP") can be used to facilitate the expression and purification of a polyketide synthase in a prokaryote such as *E.coli*. The hybrid protein can be purified, for example, using affinity chromatography using the binding protein's substrate. See, for example, *Gene* 67: 21-30 (1988). When using a fusion protein that includes maltose binding protein, a cross-linked amylose affinity chromatography column can be used to purify the protein.

The cDNA specific for a given polyketide synthase or analog thereof can also be linked using standard means to a cDNA for glutathione S-transferase ("GST"), found on a commercial vector, for example. The fusion protein expressed by such a vector construct includes the polyketide synthase or analog and GST, and can be treated for purification.

Should the MBP or GST portion of the fusion protein interfere with function, it is removed by partial proteolytic digestion approaches that preferentially attack unstructured regions, such as the linkers between MBP or GST and the polyketide synthase. The linkers are designed to lack structure, for instance using the rules for secondary structure-forming potential developed by Chou and Fasman, *Biochemistry* 13, 211, 1974. The linker is also designed to incorporate protease target amino acids, such as trypsin, arginine and lysine residues. To create the linkers, standard synthetic approaches for making oligonucleotides are employed together with standard subcloning

methodologies. Other fusion partners other than GST or MBP can also be used.

Additionally, the *Actinomadura* polyketide synthases can be directly synthesized from nucleic acid (by the cellular machinery) without use of fusion partners. For instance, nucleic acids having the sequence of any of SEQ ID NO:1-12 are subcloned into an appropriate expression vector having an appropriate promoter and expressed in an appropriate organism. Antibodies against *Actinomadura* polyketide synthases can be employed to facilitate purification.

Additional purifications techniques are applied as needed, including without limitation, preparative electrophoresis, FPLC (Pharmacia, Uppsala, Sweden), HPLC (e.g., using gel filtration, reverse-phase or mildly hydrophobic columns), gel filtration, differential precipitation (for instance, "salting out" precipitations), ion-exchange chromatography and affinity chromatography (including affinity chromatography using the RE1 duplex nucleotide sequence as the affinity ligand).

A polypeptide or nucleic acid is "isolated" in accordance with the invention in that the molecular cloning of the nucleic acid of interest, for example, involves taking an *Actinomadura* polyketide synthase gene nucleic acid from a cell, and isolating it from other nucleic acids. This isolated nucleic acid may then be inserted into a host cell, which may be yeast or bacteria, for example. A polypeptide or nucleic acid is "substantially pure" in accordance with the invention if it is predominantly free of other polypeptides or nucleic acids, respectively. A macromolecule, such as a nucleic acid or a polypeptide, is predominantly free of other polypeptides or nucleic acids if it constitutes at least about 50% by weight of the given macromolecule in a composition. Preferably, the polypeptide or nucleic acid of the present invention constitutes at least about 60% by weight of the total polypeptides or nucleic acids, respectively, that are present in a given

composition thereof, more preferably about 80%, still more preferably about 90%, yet more preferably about 95%, and most preferably about 100%. Such compositions are referred to herein as being polypeptides or nucleic acids that are 60% pure, 80% pure, 90% pure, 95% pure, or 100% pure, any of which are substantially pure.

**4. Means for Identifying Polypeptides with *Actinomadura* Polyketide Synthase Activity**

In one aspect, the present invention provides methods for identifying polypeptides that are homologous to an *Actinomadura* polyketide synthase using an *Actinomadura* polyketide synthase cDNA, for example.

Additionally, probes for *Actinomadura* polyketide synthase expression can be used, for example, to detect the presence of an *Actinomadura* polyketide synthase. Such probes include antibodies directed against an *Actinomadura* polyketide synthase or fragments thereof, nucleic acid probes that hybridize, under stringent conditions, to an *Actinomadura* polyketide synthase mRNA, and oligonucleotides that specifically prime a PCR amplification of an *Actinomadura* polyketide synthase mRNA. Nucleic acid molecules that bind to an *Actinomadura* polyketide-encoding nucleic acid under high stringency conditions are identified functionally, or by using the hybridization rules reviewed in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Press, 1989).

Many deletional or mutational analogs of nucleic acid sequences for an *Actinomadura* polyketide synthase are effective hybridization probes for *Actinomadura* polyketide synthase-encoding nucleic acid. Accordingly, the present invention relates to nucleic acids that hybridize with such *Actinomadura* polyketide synthase-encoding nucleic acids under stringent conditions. Preferably, the nucleic acid of the present

invention hybridizes, under stringent conditions, with at least a segment of any of the nucleic acids described as SEQ ID NO:1-12.

"Stringent conditions" refers to conditions that allow for the hybridization of substantially related nucleic acids, where relatedness is a function of the sequence of nucleotides in the respective nucleic acids. For instance, for a nucleic acid of 100 nucleotides, such conditions will generally allow hybridization thereto of a second nucleic acid having at least about 85% homology, and more preferably having at least about 90% homology. Such hybridization conditions are described by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Press, 1989).

PCR (polymerase chain reaction) can be used to detect nucleic acids having *Actinomadura* polyketide synthase sequences through amplification of such sequences using *Actinomadura* polyketide synthase nucleic acid primers. PCR methods of amplifying nucleic acids utilize at least two primers. One of these primers is capable of hybridizing to a first strand of the nucleic acid to be amplified and of priming enzyme-driven nucleic acid synthesis in a first direction. The other is capable of hybridizing the reciprocal sequence of the first strand (if the sequence to be amplified is single stranded, this sequence is initially hypothetical, but is synthesized in the first amplification cycle) and of priming nucleic acid synthesis from that strand in the direction opposite the first direction and towards the site of hybridization for the first primer. Conditions for conducting such amplifications, particularly under preferred high stringency conditions, are well known. See, for example, *PCR Protocols* (Cold Spring Harbor Press, 1991).

Antibodies against *Actinomadura* polyketide synthases can also be used to identify polypeptides that are homologous to *Actinomadura* polyketide synthases. Antigens for eliciting the production of antibodies against an *Actinomadura* polyketide synthase can be produced recombinantly by expressing all of or a part of the nucleic acid of an

*Actinomadura* polyketide synthase in a bacteria or a yeast or other eukaryotic cell line. In one embodiment, the recombinant protein is expressed as a fusion protein, with the non-*Actinomadura* polyketide synthase portion of the protein serving either to facilitate purification or to enhance the immunogenicity of the fusion protein. For instance, the non-*Actinomadura* polyketide synthase portion comprises a protein for which there is a readily-available binding partner that is utilized for affinity purification of the fusion protein. The antigen includes an "antigenic determinant," i.e., a minimum portion of amino acids sufficient to bind specifically with an anti-*Actinomadura* polyketide synthase antibody.

Antisera to an *Actinomadura* polyketide synthase can be made, for example, by creating an *Actinomadura* polyketide synthase antigen by linking a portion of the cDNA for *Actinomadura* polyketide synthase to a cDNA for glutathione s-transferase ("GST") found on a commercial vector. The resulting vector expresses a fusion protein containing an antigenic segment of an *Actinomadura* polyketide synthase and GST that is readily purified from the expressing bacteria using a glutathione affinity column. The purified antigenic fusion protein is used to immunize rabbits. The same approach is used to make antigens based on other segments of *Actinomadura* polyketide synthase. Procedures for making antibodies and for identifying antigenic segments of proteins are well known. See, for instance, Harlow, *Antibodies*, Cold Spring Harbor Press, 1989.

**5. Polyketides**

In addition to polyketide synthases, the present invention also provides polyketides, including purified pradimicin and pradimicin analogs, and methods for synthesizing polyketides. For example, a vector containing a nucleic acid comprising SEQ ID NO:1 can be expressed in an organism, preferably *Streptomyces*, thereby resulting in pradimicin A synthesis. Preferably, all of the polyketide synthase genes required for polyketide synthesis are present in a single vector, and the genes are preferably in the same configuration as the cDNA.

Preferred *Streptomyces* organisms for polyketide synthesis include, for example, *Streptomyces lividans*, *Streptomyces coelicor* and *Streptomyces griseus*. Preferred vectors for expression include, for example, plasmids pIJ61, pIJ702 and pIJ922, which are described in Hopwood et. al., *Gene Manipulation of Streptomyces, A Laboratory Manual* (The John Innes Foundation, Norwich, UK 1985). Preferably, the vector includes a promoter that functions well at idiophase, which is a stage of secondary metabolite production, such as the promoter of the *mel* gene, which is present in vector pIJ702.

Preferred methods for preparing a polyketide such as pradimicin or an analog thereof comprise transforming a eukaryotic or prokaryotic cell with an expression vector for expressing intracellularly or extracellularly a nucleic acid comprising a nucleic acid encoding a polypeptide sharing at least about 70% amino acid identity with an *Actinomadura* polyketide synthase, growing the transformed cell in culture, and isolating the pradimicin or analog thereof from the transformed cell or the culture medium. Preferably, the polypeptide shares at least about 80% amino acid identity with an *Actinomadura* polyketide synthase, and more preferably, the polypeptide shares at least about 90% amino acid identity with an *Actinomadura* polyketide synthase. Most preferably, the expression vector comprises a nucleic acid encoding all polyketide synthase genes necessary for synthesis of pradimicin, such as SEQ ID

NO:1. The production of pradimicin A, for example, can be detected by the presence of a red pigment. Purification of pradimicin from *Actinomadura*, for example, is described in *J. Antibiot.* 41:1701-1704 (1988).

The present invention is further exemplified by the following non-limiting example.

**Example 1. Cloning of *Actinomadura* Polyketide Synthase Genes**

Bacterial strains and plasmids

*Escherichia coli* XL1-Blue and pSE101 (*Biosci. Biotech. Biochem.* 59:1835-1841 (1995)), a shuttle cosmid vector replicable in both *Streptomyces lividans* and *E. coli,* were used for preparation of an *Actinomadura hibisca* genomic library. *E coli* XL1-Blue and plasmids pUC118 and pUC119 were used for sequencing analysis.

DNA isolation and manipulation

Plasmid and genomic DNA isolations were done by the method of Hopwood et. al., *Gene Manipulation of Streptomyces, A Laboratory Manual* (The John Innes Foundation, Norwich, UK 1985). Plasmids from *E. coli* were prepared with the Qiagen Plasmid Kit (Qiagen Inc., Chatsworth, CA). All restriction enzymes, T4 ligase and calf intestinal alkaline phosphatase were obtained from Takara (Kyoto, Japan). The procedure for library preparation is described, for example, in *Mol. Gen. Genet.* 236:39-48 (1992).

DNA hybridization

The hybridization conditions employed for reactions with the oligonucleotide probe, $^{32}$P-labeled with T4 kinase, were as follows: a Nylon membrane with immobilized DNA was prehybridized at 40°C for 4 hours in 6X SSC buffer, which contains 5X Denhardt's solution

(Maniatis et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1982)), 0.5% SDS and 100 μg/ml of heat denatured salmon sperm DNA. For overnight hybridization, the same buffer and temperature conditions were used. The genomic DNA blotted filter and plasmid DNA blotted filter were washed twice with 6X SSC buffer at 40°C for 30 minutes and with 0.6X SSC buffer at 60°C for 1 hour, respectively.

Cloning of the genes homologous to type II PKS genes

Amino acid sequences of ß-keto synthase, acyl transferase and acyl carrier protein of polyketide synthases are strongly conserved in *Streptomyces* strains producing polyketide antibiotics. See *Annu. Rev. Microbiol.* 47:875-912 (1993) and *J. Biol. Chem.* 267:19278-19290 (1992). Based on these sequences, two oligonucleotide probes were synthesized. One was designed based on the amino acid sequences of the *Streptomyces* ß-keto synthase around the cysteine residue which is thought to be an active site of the enzyme. See Figure 2, probe 1 (SEQ ID NO:16). The other probe was synthesized based on the amino acid sequences of the *Streptomyces* acyl transferase around the serine residue which is believed to be a catalytic domain. See Figure 2, probe 2 (SEQ ID NO:17). Genomic DNA from *Actinomadura hibisca* P157-2 (ATCC 53557) that was digested with several restriction enzymes was subjected to Southern blot analysis with probes 1 and 2, which were separately labeled with $^{32}P$ and then mixed. Weak but specific signals could be detected. To clone the hybridized fragment, a library was prepared from the strain P157-2 and screened by the colony hybridization with probes 1 and 2 under the same conditions as that for genomic Southern analysis. Several positive cosmid clones were found to hybridize to the probes. Two clones, designated pPRM1 and pPRM14, were selected for further analysis.

The physical maps of pPRM1 and pPRM14 were determined and are shown in Figure 3. Using Southern blot hybridization analysis of chromosomal DNA of the strain P-157-2 with these two cosmid clones as probes, it was confirmed that the inserted DNAs of pPRM1 and pPRM14 had not been structurally rearranged during the construction of the library. The position of the hybridized region with oligonucleotide probes was defined by Southern blot analysis.

Sequence analysis.

The 8.2-kb *Sac*I fragment prepared from pPRMI was cloned into the *Sac*I sites of pUC118 and pUC119 (pUC118 and pUC119 are available, for example, from Takara Syuzo, Kyoto, Japan). After construction of a series of plasmids subcloned from these plasmids, single stranded DNAs were prepared with helper phage M13 KO7, which is also available, for example, from Takara Syuzo. Sequencing was done by the dideoxy chain termination method of Sanger *et al.*, using an automatic DNA sequencer ALF (Pharmacia, Sweden). It was also done with [$\alpha$-$^{35}$S]-dCTP as the radioactive label.

Nucleotide sequence of the DNA fragment hybridized to the probe

As one approach to examine whether the DNA fragment hybridized to the probes carries the PKS gene for biosynthesis of PRM A, the nucleotide sequence of the 8.2-kb *Sac*I fragment containing hybridized region was determined. Computer analysis of the DNA sequence, using Frame Analysis (See *Gene* 30:157-166 (1984)), revealed eleven ORFs (ORF1-11), which are oriented in the same direction except for ORF10. To understand the functions of each the ORFs deduced by DNA sequencing, databases, including DNASIS, were searched using their translated products. The results are summarized in Table 1, *infra*. The ORF1, ORF2 and ORF3 gene products show strong similarities (44-73% amino acid identity) with ORF 1, 2 and 3 gene

products of *gra* (*EMBO J.* 8:2717-2725 (1989)), *tcm* (*EMBO J.* 8:2727-2736 (1989)) and *act* (*J. Biol. Chem.* 267:19278-19290(1992)), which are known to encode condensing enzyme, acyltransferase and acyl carrier protein for granaticin, tetracenomycin and actinorhodin biosynthesis, respectively. The proteins encoded by ORF4 and ORF6 have similarities with the N and C-terminal half of the TcmN protein (*J. Bacteriol.* 174:1810-1820 (1992)) (52% and 46% amino acid identity), respectively, which is thought to be a multifunctional cyclase/dehydratase participating in tetracenomycin biosynthesis. The ORF7 gene product is homologous to the *fab*G product of *E coli* (*J. Biol. Chem.* 267:5751-5754 (1992)) (3-ketoacyl-ACP reductase, 38% amino acid identity) and granaticin-producing polyketide synthase chains 5 and 6 (*EMBO J.* 8:2717-2725 (1989)) (30% and 35% amino acid identity, respectively). Both of the ORF8 and ORF9 gene products have some similarity to hypothetical protein 1 participating in spore color formation in *Streptomyces coelicolor* (*Mol. Microbiol.* 4:1679-1691 (1990)) (23 and 24% amino acid identity, respectively) in a limited region. The ORF10 gene product has a significant similarity to a variety of monooxygenases, including cytochrome P450 (28-40% amino acid identity). The ORF11 gene product shows similarity with the hypothetical protein 1 participating in spore color formation in *Streptomyces coelicolor* (*Mol. Microbiol.* 4:1679-1691 (1990)) (51% amino acid identity), and less extensive, although significant, with the CurG protein of *Streptomyces cyaneus* (*Gene* 117:131-136 (1992)) (45% amino acid identity) and the *tcm*I protein of *Streptomyces glaucescens* (EMBL data library no. S27691) (35% amino acid identity). The ORF5 gene product shows some similarity to a histidine kinase of *Caulobacter crescentus* (*Proc. Natl. Acad. Sci.* 89:10297-10301 (1992)) and multicatalytic endopeptidase of *S. cerevisiae* (*Mol. Cell. Biol.* 11:344-353 (1991)).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Oki, Toshikazu
Dairi, Tohru

(ii) TITLE OF INVENTION: POLYKETIDE SYNTHASES FOR PRADIMICIN BIOSYNTHESIS AND DNA SEQUENCES ENCODING SAME

(iii) NUMBER OF SEQUENCES: 25

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 29,135
(C) REFERENCE/DOCKET NUMBER: BMS-X25

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8169 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAGCTCGGCC ACGTCGACAC CGAGGAGCTG CCCGCCCCCG ACGAGCAGGG GCTCGACGTC 60

GGGGGCCGCA CGTGAGCGGA CCGCAGGGGG GCGGGCCGCG CCGCCGTGCG ATCACCGGCA 120

TGGGGGTGGT CGCGCCCGGC GGCTCGGGCC GGAAGGCGTT CTGGAACCTG CTGACCGACG 180

GCCGCACCGC GACCCGGAAG ATCTCGCTGT TCGACCCGGC GGGCTTCCGG TCCCGGATCG 240

CCGCCGAGTG CGACTTCGAC CCCGCCGCCG AGGGGCTGAC GCCCCGCGAG GTCCGGCGCA 300

TGGACCGGGC CGCGCAGCTC GCGGTGGTGT CGGCGCGCGA GGCGCTCGCC GACAGCGGGC 360

TGGGGGCGGG CGAGGGCGAC CCGGCGCGGT TCGCGGTGTC GCTCGGCAGC GCCGTCGGCT 420

GCACGATGGG GCTGGAGGAC GAGTACGTCG TGGTCAGCGA CCAGGGCCGC GACTGGCTGG 480

TCGACCACTC CTACGGCGTG CCGCACCTGT ACCGGCACCT GGTGCCCAGC TCGCTGGCGG 540

CCGAGGTCGC CTGGGCGGGC GGGGCCGAGG GCCCGGTCAC GCTGATCTCG ACGGGCTCGA 600

CCTCCGGGCT CGACGCGGTC GGGCACGGCG CGCGCGTCAT CGCCGAGGGC TCGGCGGACG 660

TGGCGCTCGC CGGGGCCACC GACGCGCCCA TCTCGCCGAT CACGGTGGCG TGCTTCGACG 720

CCATCCGGGC GACCTCGCCG AACAACGACG ACCCCGAGCA CGCGTCCCGG CCGTTCGACC 780

GGGAGCGCAA CGGGTTCGTG CTCGGCGAGG GCGCGGCGGT GTTCGTCCTG GAGGAGCTGG 840

AGCACGCCCG CCGCCGGGGC GCGCACGTCT ACTGCGAGGT CGCGGGGTAC GCCACGCGCG 900

GCAACGCCTA CCACATGACG GGCCTGAAGC CCGACGGCCG CGAGATGGCC GAGGCGATCA 960

GGGTGGCGAT GGACGCCGCC CGGGTCGCCC CGGCCGACCT CGACTACATC AACGCGCACG 1020

GCTCGGGCAC CAAGCAGAAC GACCGGCACG AGACGGCCGC GTTCAAGCGC AGCCTCGGCG 1080

AGCGCGCCTA CGAGCTGCCG GTCAGCTCCA TCAAGTCGAT GGTCGGGCAC TCGCTCGGCG 1140

CGATCGGCTC GATCGAGCTG GCCGCGTGCG CGCTGGCGAT CGAGCACGGT GTGGTGCCGC 1200

CGACCGCCAA CCTGCACAAC GCCGACCCCG AATGCGACCT GGACTACGTG CCGCTGGTGG 1260

CGCGCGAGGG CCCGATCCGC ACGGTGCTGA GCGTGGGCAG CGGCTTCGGC GGCTTCCAGT 1320

CCGCCACCGT CCTGCGGGAG GCCGCGTGAG CGTCCTGACG GCGGACGCGC CGGCGGTCAC 1380

CGGGATCGGC GTGGTCGCGC CGACCGGGAT CGGCGTCGAG GAGCACTGGG CGGCGACGTT 1440

GCGCGGCGTC CCGGTCATCG GGCCGCTGAC CAGGTTCGAC GCCGCGCGCT ACCCGTCGCC 1500

GTTCGGCGGC GAGGTGCCCG GGTTCGACGC CGCCGAGCGC GTCCCGGGGC GGCTCATCCC 1560

GCAGACCGAC CACTGGACGC ACCTGGCGCT GGCCGCCACC GACCTCGCCC TCGCCGACGC 1620

GGGCGTGGTC CCGGCCGAGC TGCCCGAGTA CGAGATGGCG GTGGTGACCG CCAGCTCGTC 1680

GGGCGGCGTG GAGTTCGGGC AGCGCGAGAT CCAGGCGTTG TGGCGGGACG GGCCCCGGCA 1740

CGGCGGGGCC TACCAGTCGA TCGCCTGGTT CTACGCGGCG ACGACCGGCC AGATCTCCAT 1800

CCGGCACGGG ATGCGCGGCC CCTGCGGCGT CGTGGTCGCC GAGCAGGCCG GGGCGCTGGA 1860

GTCGTTCGCG CAGGCCCGCC GCTACCTGGC GGACGGGGCG CGGGTGGTGG TGTCCGGCGG 1920

CACCGACGCG CCGTTCAGTC CGTACGGCCT GACCTGCCAG CTCGGCAGCG GGCGGCTTAG 1980

CACGGGTGCC GACCCGGCCC GCGCCTACCT GCCGTTCGAC GCCGCCGCGA ACGGCTTCGT 2040

GCCGGGCGAG GGCGGCGCGA TCCTCATCAT CGAGCAAGCC GCCACCGCGC AGGACCGCTC 2100

CTACGGGCGG ATCGCGGGCT ACGCGGCGAC CTTCGACCCG CCGCCGGGCT CGGGCCGCCC 2160

TCCGACGCTG GAGCGAGCCG TGCGCGCCGC CTTGGACGAC GCCCGGCTCA CACCCGCCGA 2220

CGTGGACGTG GTGTTCGCCG ACGCGGCGGG CGTCCCGGAT CTGGACCGCG CGGAGGCCGA 2280

CGCGATCGGC GCGGTCTTCG GGCCGCGCGG CGTGCCCGTC ACCGCGCCCA AGAGCCTGAC 2340

CGGCCGCCTG TACGCGGGCG GCCCCGCGCT CGACGCCGCG ACGGCGCTGC TGGCCATGCA 2400

CGACTCGGTG ATCCCGCCGA CGGCCGGCGG CGCGGACGTC CCGCCCGGCT ACGCGCTCGA 2460

CCTGGTCGGC GCGGAACCGC GCCCGGCCCG GCTGCGCACC GCACTGATCA TCGCCCGCGG 2520

CTACGGGGGC TTCAACGCCG CCCTGGTGCT GCGCGGCCCG AACACCTGAC AACGACCCGA 2580

GAGGACGGAC GAGATGGCAA CCCGCGAACG CACCATCGAC GACCTGCGCG CGCTGATGCG 2640

CGCCGCCGTC GGCGAGGCCG ACGACATCGA CCTGGACGGC GACATCCTCG ACTCCACCTT 2700

CACCGAGCTG GAGTACGACT CGCTCGCCGT GCTGGAGCTC GCGGCCCGCA TCGAGACGCA 2760

GTGGGGCGTG CTGATCCCCG AGGACGACGC GTCCGGGCTG GAGACCCCGC GCATGTTCCT 2820

CGACTACGTG AACGGGCGGG CGGTGGCCGA GCGATGACGC AGTGGCGCAC CGACAGCGTG 2880

ATCGTGATCG ACGCGCCGCT CGACGTCGTC TGGGACATGA CCAACGACGT CGCCTCCTGG 2940

CCGGAGCTGT TCGACGAGTA CGCCTCGGCC GAGATCCTGG AGCGCGACGG CGACACCGTC 3000

CGCTTCCGGC TGACGATGCA CCCCGACGCC GACGGCAACG CCTGGTCGTG GGTGTCGGAG 3060

CGCACGCCCG ACCGCGCCGC GCTCACCGTC AACGCGCACC GCGTGGAGAC CGGCTGGTTC 3120

GAGCACATGA ACCTGCGCTG GGACTACCGC GAGGTGCCCG GCGGCGTGGA GATGCGCTGG 3180

CGGCAGGACT TCGCGATGAA GGAGGCGTCG CCGGTGTCGC TGGCGGCGAT GACCGAGCGC 3240

ATCCAGAGCA ACTCCCCCGT CCAGATGAAG CTGATCAAGG ACAAGGTGGA GCGGGCGGCC 3300

CGGGGCGCGC GGTGATCGAG TTCCTGCTCC CGGTCGCGCT GCTCGGCAAC GGGTTGTGCG 3360

CGGGCGTGCT GACGGGCAGC GTCCTCGGCG TCGTGCCGTA CTACCGGACG CTGCCCGAGG 3420

ACCGCTACAT CGCCGCGCAC GCCTTCGCGG TCGGCCGCTA CGACCCGTTC CAGCCGGTGT 3480

GCCTGCTGGT CACGGTGGCG GCCGACGCGG TCGCGGCGGC GGTCGCGCCG ACCGCCGCCG 3540

CCCGGGTGCT CTGCGCGCTC GCCGCCGTGC TCGCGCTGGC GGTGGTGGCG ATCTCGCTCA 3600

CCCGCAACGT GCCGATGAAC CGCCGGATCA AGCGGCTGGA CCCGGCCGCG CCGCCCGCCG 3660

GGTTCAGCGC GCCCGCGTTC CTGCGCCGCT GGGCGGGCTG GAACGCGGCG CGCACCGGCC 3720

TGACGCTGGC CGCCCTTCTC AGCAACACGG CCGCCCTCGG CGTGCTGCTG TGACCGATCG 3780

GGAAGGGAGG GACATGACCG AACCGGAAGG ACCGCACGCC GCGAGCCTGC GGCTCCAATC 3840

TCTGCTGGAC GGCATGCGCG TCGCCAAGGT CGTCCAGGTG CTCGCCGAAC TCCAGGTGGC 3900

CGACGCGGTC GCCGACGGCC CCTGCAAGCC CGCCGAGATC GCCGCCGACG TCGGCGCCGA 3960

CCCCGACGCG CTGTACCGGG TGCTGCGCTG CGCCGCCTCG TTCGGGGTGT TCACCGAGGA 4020

CGAGGACGGC CGGTTCGGGC TCACCCCGAT GCCCGCGCTG CTGCGCACCG GCACCGACGA 4080

CAGCCACCGC GACCTGTTCA TGATGGCGGC GGGCGACCTG TGGTGGCGGC CGTACGGCGA 4140

GCTGCTGGAG ACGGTGCGGA CCGGCCGCCC CGCCGCCGAG CTGGCGTTCG GGATGCCGTT 4200

CTACGACTAC CTCGGCACCG ACCCGGCCGC CGCCGGGCTC TTCGACCGCG CGATGACGCA 4260

GGTCAGCAAG GGCCAGGCGA AGGCGATCCT CGGCCGCTGC TCGTTCGAGC GGTACGCGCG 4320

GATCGCCGAC GTGGGCGGCG GCCACGGCTA CTTCCTCGCG CAGGTGTTGC GCAGCAGCCC 4380

GCGCACCGAG GGCGTGCTGC TGGACCTGCC GCACGTGGTG GCCGGAGCCC CGGCGGTGCT 4440

GGAGAAGCAC GAGGTCGCCG ACCGCGTCCA GGTCGTCCCG GGCAGCTTCT TCGACGCGCT 4500

GCCCACCGGC TGCGACGCCT ACCTGCTGAA AGCGATCCTC ATCAACTGGC CCGACGCCGA 4560

CGCCGAACGC ATCCTGCACC GGGTGCCGCA GGCGATCGGC AACGACCGCG ACGCGCGGCT 4620

GCTGGTGGTC GAGCCCGTCG TCCCGCCCGG CGACGTCCGC GACTACAGCA AGGCCACCGA 4680

CATCGACATG CTCGCCATCA TCGGCGGGCG GCAGCGCACC GTCGCCGAGT GGCGGCGGCT 4740

GCTGCGCGCG GGCGGCTTCG AGCTGGTGGG CGAGCCCACG CCGGGCCGCC GCGAGGTCAT 4800

GGAGTGCCGC CCCATCTGAA CCCGTCCCAC CCGTCGCCCA CATCCAGGGA GAACGCATGA 4860

CCGACACATC GTTCGCCGGC AAGAACGCGC TGATCACCGG CGGCACCCGG GGCATCGGCC 4920

GGGCCGTCGC GCTCGGCCTG GCCGGCGCCG GGGCCAATGT CACCGTCTGC TACCGCAGCG 4980

ACGCCGAGTC CGCCGCCGCG ATGGAAGCCG AGCTGGCCGC CACCGACGGC AAGCACCACG 5040

TCCTCCAGGC CGACATCGGC AACGCCGGGG ACGTCCGCCG CCTGCTGGAC GAGGTCGCCG 5100

CCCGCATGGG CTCGCTCGAC GTAGTCGTGC ACAACGCCGG GCTGATCAGC CACGTGCCGT 5160

TCGCCGACCT GGAGCCCGAG GAGTGGCACC GGATCGTCGA CTCCAACCTG ACCGGCATGT 5220

ACCTGGTGGT GCGGGCCGCG CTGCCGCTGC TGTCGGAGGG CGGCGCGGTC GTCGGCGTCG 5280

GCTCCAAGGT CGCGCTCGTC GGCATCTCGC AGCGCACCCA CTACACCGCC GCCAAGGCCG 5340

GGCTCATCGG GTTCGTGCGC TCGCTCAGCA AGGAGCTGGG GCCGCTCGGC ATCCGGGTCA 5400

ACCTGGTCGC GCCCGGCATC ACCGAGACCG ACCAGGCCGC GCACCTGCCC CCCGTGCAGC 5460

GCGAGCGCTA CCAGAGCATG ACCGCGCTCA AGCGGCTCGG CCAGGCCGAC GAGGTCGCCG 5520

ACGTGGTGCT GTTCCTCGCC GGTCCCGGCG CGCGCTACGT CACCGGCGAG ACCGTCAACG 5580

TGGACGGGGG GATGTGACCA TGGCCGACAG CGGCCCGGTG TTCCGGGTGA TGCTCCGGAT 5640

GGAGATCGTC CCGGGCAGGG AGGCGGAGTT CGAGCGGGTC TGGTACTCGG TCGGCGACAC 5700

CGTCAGCGGC AACCCCGCCA ACCTCGGCCA GTGCGTGCTG CGCAGCGACG ACGAGGAGAG 5760

CGTCTACTAC ATCATGAGCG ACTGGATCGA CGAGGCGCGG TTCCGCGAGT TCGAGCGCAG 5820

CGACGGCCAC GTCTAGCACC GCCGCAAGCT GCACCCGTAC CGGGTGAAGG GCAGCATGGC 5880

GACGATGAAG GTCGTGCACG ACCTCGGCCG CGCGGCGGCG GAGCCGGTCC GGTGACGGCC 5940

GGGCAGGTGC GGGTCCTGGT CCGCTACCAG GCTCCGGGCG ACGACCCCGA GGCCGTCGTC 6000

CAGGCGTACA AGCTGGTCTG CGAGGAACTG CGCGGGACGC CCGGCCTGCT CGGCAGCGAG 6060

CTGCTGGCGT CGCACGCTCG ACGAGGGACG GTTCGCGGTG CTGAGCCTGT GGAGCGACGC 6120

CGCGCGGTTC CAGGAATGGG AGCAGGGCCC GGCGCACAAG GGCCAGACGT CCGGCCTGCG 6180

CCCGTTCCGG GACACCTCTT CGGGGCGCGG CTTCGATTTC TACGAAGTGG TGCACGCCCT 6240

GTAAGAACAA CGAAGGGCCC GGCACGCGCA TGGCGTGCCG GGCCCTTTCA CATCCGTGCC 6300

TACCAGGCGA TGGGCAGCGC GTCCGGCCGC GCGAACGCCA AGCCGGGCCG CCAGGTGATG 6360

TCGGCATCGT CGATAGCGAG ACGCAGCGCG GGCGTCCGCT CCACCAGCGT CTCCAGCACG 6420

ACCTGAAGCT CCAGCCGGGC GAGCGGCGCG CCCAGGCAGT AGTGGATGCC GTGGCCGAGC 6480

GCGATGTGCG GGTTGTCGGT ACGGCCGAGG TCGAGTTCCT CGGGATCGGC GAACACCTCC 6540

GGATCGCGGT TGGCGGCGTT GAAAAGCGGG ATGACCGCCT CGCCCGCGCG CACGAGGGTG 6600

CCGCCGACTT CCACATCCTC GACCGCGATG CGGATCGCGC CCGCGCCGCC GCCGATCTGC 6660

CCGTACCGTA GCAGTTCCTC AACGGCCGCC GGGATACCCG ACGGGTCCTC GCGCAGCCGC 6720

GCGTACCGCG ACGGCTCGCG CAGCAGGTGG TAGACCGAGT GCGTGATCGC CGCCGTGGTG 6780

GTGTGGTAAC CCGCCGCCAG CAGCGTCATG CCGAAGGTGA GCAGTTCCTC CTCGCTGAGG 6840

CCGTCGTCGG CGTGCGCCGG GCTCAGCAAC GACAGCAGGT CGTCGGCGGG CGCGGCCGTC 6900

TTGGCGTCGA TCAGCTCGGC GAGGTAGCCG CGCAGCCGCC CGACCGCGGC CTTGATCTCG 6960

TCGGCCTGCG CGAGAGCGGG CGCGCCGATG GTGAGCATCC GGTCGGTCCA GTCCTGGAAG 7020

CGCGGCCGAT CCTCCGGCGG AACGCCCAGC ATCTCGCAGA TGACGGTGAC CGGCAGCGGC 7080

AGCGCCAGGT GCGCGATCAG GTCGGCGGGC GGGCCGTGCT CGACCATCTC GTCCACGAAC 7140

CCCGACGTCA GGTCGCGCAC GTGCGCGCGC ATCCCCTCCA CACGACGGGC GGTGAACGCG 7200

CGAGACACGA TCTTGCGCAT CCTCGTGTGC TCGGGCGGGC TCATGATGAC CAGCGACTTG 7260

GAGCCGCGCT GCATCGGGAT CAGGCGCGGC GCGCCCGGCC GGGTCACCGC CTCCTTGCTG 7320

AAGCGCCGGT CCGAGGTGAC GAACCGGACG CTGGCGTAGC GCGTCACGAC CCACGCGTGG 7380

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TCGCCGGTCG GCAGCACCAC CTTGGCGACC GGGTCGGACG CGCGCAGGCG CGCGTGCTCG 7440

CACGGCGGCT GGAAGGGGTC GTCCGGCCGG AACGGGAAGG CCGGCGTGAC GTCGGGGCGG 7500

GGGTCGACGG TCGGGGCATC CTTCGAGGAG GGCATACGCC AGGCTTGCAA GGACGCCTCG 7560

AAGCGGGCTC AACGCGGGCT CGCTCCACCG TCCTTCGAGC GGCCCCCGAG CTGCGGTGAC 7620

CACACTCTGC GGCTACCGGC TCACAGCCCC GACCGAGGGA TGGTTCCCAT GGACAGGTTC 7680

CTGATCGTCG CCCGCATGTC CCCCTCGTCG GAGAAGGAGG TGGCGCGCCT GTTCGCCGAG 7740

TCCGACGAGG GCACCGAGCT GCCGGAGGTG GCCGGGACGG TCAGCCGCAG CCTGCTGTCG 7800

TTCCACGGCC TGTACTTCCA CCTGACGGAG GTGGAGGAGA GCACGGACAG GACGCTCAAC 7860

GGCATCCACG AACACCCCGA GTTCGTCCGG CTGAGCCGCC AGCTGTCCGG TCACGTCCAG 7920

GCGTACGACC CGAAGACGTG GCGCTCGCCC GCCGACGCCA TGGCCCGCGA GTTCTACCGG 7980

TGGGAGGCGG GGACCGGCGT CGTGCGCCGC TGACCCGTCC CGAGTCCCAC CGGTCGCAGG 8040

TTCGTCACTC TCCGTTGACT CCCTTCCTCG ATAGCGTCAT CGTTGGTGGC CCACCTGGAC 8100

GACGGAGCCA TCTGAGGGGA AGCGTTGGGT ACCGATACTC TCCCGAGACT CACCGACGCC 8160

GGAGAGCTC 8169

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1278 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTGAGCCGAC CGCAGGGGGG CGGGCCGCGC CGCGTCGCGA TCACCGGCAT GGGGGTGGTC 60

GCGCCCGGCG GCTCGGGCCG GAAGGCGTTC TGGAACCTGC TGACCGACGG CCGCACCGCG 120

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ACCCGGAAGA TCTCGCTGTT CGACCCGGCG GGCTTCCGGT CCCGGATCGC CGCCGAGTGC 180

GACTTCGACC CCGCCGCCGA GGGGCTGACG CCCCGCGAGG TCCGGCGCAT GGACCGGGCC 240

GCGCAGCTCG CGGTGGTGTC GGCGCGCGAG GCGCTCGCCG ACAGCGGGCT GGTGGCGGGC 300

GAGGGCGACC CGGCGCGGTT CGCGGTGTCG CTCGGCAGCG CCGTCGGCTG CACGATGGGG 360

CTGGAGGACG AGTACGTCGT GGTCAGCGAC CAGGGCCGCG ACTGGCTGGT CGACCACTCC 420

TACGGCGTGC CGCACCTGTA CCGGCACCTG GTGCCCAGCT CGCTGGCGGC CGAGGTCGCC 480

TGGGCGGGCG GGGCCGAGGG CCCGGTCACG CTGATCTCGA CGGGCTGCAC CTCCGGGCTC 540

GACGCGGTCG GGCACGGCGC GCGCGTCATC GCCGAGGGCT CGGCGGACGT GGCGCTCGCC 600

GGGGCCACCG ACGCGCCCAT CTCGCCGATC ACGGTGGCCT GCTTCGACGC CATCCGGGCG 660

ACCTCGCCGA ACAACGACGA CCCCGAGCAC GCGTCCCGGC CGTTCGACCG GGAGCGCAAC 720

GGGTTCGTGC TCGGCGAGGG CGCGGCGGTG TTCGTCCTGG AGGAGCTGGA GCACGCCCGC 780

CGCCGGGGCG CGCACGTCTA CTGCGAGGTC GCGGGGTACG CCACGCGCGG CAACGCCTAC 840

CACATGACGG GCCTGAAGCC CGACGGCCGC GAGATGGCCG AGGCGATCAG GGTGGCGATG 900

GACGCCGCCC GGGTCGCCCC GGCCGACCTC GACTACATCA ACGCGCACGG CTCGGGCACC 960

AAGCAGAACG ACCGGCACGA GACGGCCGCG TTCAAGCGCA GCCTCGGCGA GCGCGCCTAC 1020

GAGCTGCCGG TCAGCTCCAT CAAGTCGATG GTCGGGCACT CGCTCGGCGC GATCGGCTCG 1080

ATCGAGCTGG CCGCGTGCGC GCTGGCGATC GAGCACGGTG TGGTGCCGCC GACCGCCAAC 1140

CTGCACAACG CCGACCCCGA ATGCGACCTG GACTACGTGC CGCTGGTGGC GCGCGAGGGC 1200

CGCATCCGCA CGGTGCTGAG CGTGGGCAGC GGCTTCGGCG GCTTCCAGTC CGCCACCGTC 1260

CTGCGGGAGG CCGCGTGA 1278

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1223 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTGAGCGTCC TGACGGCGGA CGCGCCGGCG GTCACCGGGA TCGGCGTGGT CGCGCCGACC 60

GGGATCGGCG TCGAGGAGCA CTGGGCGGCG ACGTTGCGCG GCGTCCCGGT CATCGGGCCG 120

CTGACCAGGT TCGACGCCTC GCGCTACCCG TCGCCGTTCG GCGGCGAGGT GCCCGGGTTC 180

GACGCCGCCG AGCGCGTCCC GGGGCGGCTC ATCCCGCAGA CCGACCACTG GACGCACCTG 240

GCGCTGGCCG CCACCGACCT CGCCCTCGCC GACGCGGGCG TGGTCCCGGC CGAGCTGCCC 300

GAGTACGAGA TGGCGGTGGT GACCGCCAGC TCGTCGGGCG GCGTGGAGTT CGGGCAGCGC 360

GAGATCCAGG CGTTGTGGCG GGACGGGCCC CGGCACGTCG GGGCTACCAG TCGATCGCCT 420

GGTTCTACGC GGCGACGACC GGCCAGATCT CCATCCGGCA CGGGATGCGC GGCCCCTGCG 480

GCGTCGTGGT CGCCGAGCAG GCCGGGGCGC TGGAGTCGTT CGCGCAGGCC CGCCGCTACC 540

TGGCGGACGG GGCGCGGGTG GTGGTGTCCG GCGGCACCGA CGCGCCGTTC AGTCCGTACG 600

GCCTGACCTG CCAGCTCGGC AGCGGGCGGC TTAGCACGGG TGCCGACCCG GCCCGCGCCT 660

ACCTGCCGTT CGACGCCGCC GCGAACGGCT TCGTGCCGGG CGAGGGCGGC GCGATCCTCA 720

TCATCGAGCA AGCCGCCACC GCGCAGGACC GCTCCTACGG GCGGATCGCG GGCTACGCGG 780

CGACCTTCGA CCCGCCGCCG GGCTCGGGCC GCCCTCCGAC GCTGGAGCGA GCCGTGCGCG 840

CCGCCTTGGA CGACGCCCGG CTCACACCCG CCGACGTGGA CGTGGTGTTC GCCGACGCGG 900

CGGGCGTCCC GGATCTGGAC CGCGCGGAGG CCGACGCGAT CGGCGCGGTC TTCGGGCCGC 960

GCGGCGTGCC CGTCACCGCG CCCAAGAGCC TGACCGGCCG CCTGTACGCG GGCGGCCCCG 1020

CGCTCGACGC CGCGACGGCG CTGCTGGCCA TGCACGACTC GGTGATCCCG CCGACGGCCG 1080

GCGGCGCGGA CGTCCCGCCC GGCTACGCGC TCGCCCTGGT CGGCGCGGAA CCGCGCCCGG 1140

CCCGGCTGCG CACCGCACTG ATCATCGCCC GCGGCTACGG GGGCTTCAAC GCCGCCCTGG 1200

TGCTGCGCGG CCCGAACACC TGA 1223

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 264 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATGGCAACCC GCGAACGCAC CATCGACGAC CTGCGCGCGC TGATGCGCGC CGCCGTCGGC 60

GAGGCCGACG ACATCGACCT GGACGGCGAC ATCCTCGACT CCACCTTCAC CGAGCTGGAG 120

TACGACTCGC TCGCCGTGCT GGAGCTCGCG GCCCGCATCG AGACGCAGTG GGGCGTGCTG 180

ATCCCCGAGG ACGACGCGTC CGGGCTGGAG ACCCCGCGCA TGTTCCTCGA CTACGTGAAC 240

GGGCGGGCGG TGGCCGAGCG ATGA 264

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 462 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGACGCAGT GGCGCACCGA CAGCGTGATC GTGATCGACG CGCCGCTCGA CGTCGTCTGG 60

GACATGACCA ACGACGTCGC CTCCTGGCCG GAGCTGTTCG ACGAGTACGC CTCGGCCGAG 120

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ATCCTGGAGC GCGACGGCGA CACCGTCCGC TTCCGGCTGA CGATGCACCC CGACGCCGAC 180

GGCAACGCCT GGTCGTGGGT GTCGGAGCGC ACGCCCGACC GCGCCGCGCT CACCGTCAAC 240

GCGCACCGCG TGGAGACCGG CTGGTTCGAG CACATGAACC TGCGCTGGGA CTACCGCGAG 300

GTGCCCGGCG GCGTGGAGAT GCGCTGGCGG CAGGACTTCG CGATGAAGGA GGCGTCGCCG 360

GTGTCGCTGG CGGCGATGAC CGAGCGCATC CAGAGCAACT CCCCCGTCCA GATGAAGCTG 420

ATCAAGGACA AGGTGGAGCG GGCGGCCCGG GGCGCGCGGT GA 462

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 462 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTGATCGAGT TCCTGCTCCC GGTCGCGCTG CTCGGCAACG GGTTGTGCGC GGGCGTGCTG 60

ACGGGCAGCG TCCTCGGCGT CGTGCCGTAC TACCGGACGC TGCCCGAGGA CCGCTACATC 120

GCCGCGCACG CCTTCGCGGT CGGCCGCTAC GACCCGTTCC AGCCGGTGTG CCTGCTGGTC 180

ACGGTGGCGG CCGACGCGGT CGCGGCGGCG GTCGCGCCGA CCGCCGCCGC CCGGGTGCTC 240

TGCGCGCTCG CCGCCGTGCT CGCGCTGGCG GTGGTGGCGA TCTCGCTCAC CCGCAACGTG 300

CCGATGAACC GCCGGATCAA GCGGCTGGAC CCGGCCGCGC CGCCCGCCGG GTTCAGCGCG 360

CCCGCGTTCC TGCGCCGCTG GGCGGGCTGG AACGCGGCGC GCACCGGCCT GACGCTGGCC 420

GCCCTGCTCA GCAACACGGC CGCCCTCGGC GTGCTGCTGT GA 462

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 1026 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGACCGAAC CGGAAGGACC GCACGCCGCG AGCCTGCGGC TCCAATCTCT GCTGGACGGC 60

ATGCGCGTCG CCAAGGTCGT GCAGGTGCTC GCCGAACTCC AGGTGGCCGA CGCGGTCGCC 120

GACGGCCCCT GCAAGCCCGC CGAGATCGCC GCCGACGTCG GCGCCGACCC CGACGCGCTG 180

TACCGGGTGC TGCGCTGCGC CGCCTCGTTC GGGGTGTTCA CCGAGGACGA GGACGGCCGG 240

TTCGGGCTCA CCCCGATGGC CGCGCTGCTG CGCACCGGCA CCGACGACAG CCACCGCGAC 300

CTGTTCATGA TGGCGGCGGG CGACCTGTGG TGGCGGCCGT ACGGCGAGCT GCTGGAGACG 360

GTGCGGACCG GCCGCCCCGC CGCCGAGCTG GCGTTCGGGA TGCCGTTCTA CGACTACCTC 420

GGCACCGACC CGGCCGCCGC CGGGCTCTTC GACCGCGCGA TGACGCAGGT CAGCAAGGGC 480

CAGGCGAAGG CGATCCTCGG CCGCTGCTCG TTCGAGCGGT ACGCGCGGAT CGCCGACGTG 540

GGCGGCGGCC ACGGCTACTT CCTCGCGCAG GTGTTGCGCA GCAGCCCGCG CACCGAGGGC 600

GTGCTGCTGG ACCTGCCGCA CGTGGTGGCC GGAGCCCCGG CGGTGCTGGA GAAGCACGAG 660

GTCGCCGACC GCGTCCAGGT CGTCCCGGGC AGCTTCTTCG ACGCGCTGCC CACCGGCTGC 720

GACGCCTACC TGCTGAAAGC GATCCTCATC AACTGGCCCG ACGCCGACGC CGAACGCATC 780

CTGCACCGGG TGCGCGAGGC GATCGGCACC GACCGCGACG CGCGGCTGCT GGTGGTCGAG 840

CCCGTCGTCC CGCCCGGCGA CGTCCGCGAC TACAGCAAGG CCACCGACAT CGACATGCTC 900

GCCATCATCG GCGGGCGGCA GCGCACCGTC GCCGAGTGGC GGCGGCTGCT GCGCGCGGGC 960

GGCTTCGAGC TGGTGGGCGA GCCCACGCCG GGCCGCCGCG AGGTCATGGA GTGCCGCCCC 1020

ATCTGA 1026

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 741 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGACCGACA CATCGTTCGC CGGCAAGAAC GCGCTGATCA CCGGCGGCAC CCGGGGCATC 60

GGCCGGGCCG TCGCGCTCGG CCTGGCCCGC GCCGGGGCCA ATGTCACCGT CTGCTACCGC 120

AGCGACGCCG AGTCCGCCGC CGCGATGGAA GCCGAGCTGG CCGCCACCGA CGGCAAGCAC 180

CACGTGCTCC AGGCCGACAT CGGCAACGCC GGGGACGTCC GCCGCCTGCT GGACGAGGTC 240

GCCGCCCGCA TGGGCTCGCT CGACGTAGTC GTGCACAACG CCGGGCTGAT CAGCCACGTG 300

CCGTTCGCCG ACCTGGAGCC CGAGGAGTGG CACCGGATCG TCGACTCCAA CCTGACCGGC 360

ATGTACCTGG TGGTGCGGGC CGCGCTGCCG CTGCTGTCGG AGGGCGGCGC GGTCGTCGGC 420

GTCGGCTCCA AGGTCGCGCT CGTCGGCATC TCGCAGCGCA CCCACTACAC CGCCGCCAAG 480

GCCGGGCTCA TCGGGTTCGT GCGCTCGCTC AGCAAGGAGC TGGGGCCGCT CGGCATCCGG 540

GTCAACCTGG TCGCGCCCGG CATCACCGAG ACCGACCAGG CCGCGCACCT GCCCCCCGTG 600

CAGCGCGAGC GCTACCAGAG CATGACCGCG CTCAAGCGGC TCGGCCAGGC CGACGAGGTC 660

GCCGACGTGG TGCTGTTCCT CGCCGGTCCC GGCGCGCGCT ACGTCACCGG CGAGACCGTC 720

AACGTGGACG GGGGGATGTG A 741

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 342 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTGACCATGG CCGACAGCGG CCCGGTGTTC CGGGTGATGC TCCGGATGGA GATCGTCCCG 60

GGCAGGGAGG CGGAGTTCGA GCGGGTCTGG TACTCGGTCG GCGACACCGT CAGCGGCAAC 120

CCCGCCAACC TCGGCCAGTG CGTGCTGCGC AGCGACGACG AGGAGAGCGT CTACTACATC 180

ATGAGCGACT GGATCGACGA GGCGCGGTTC CGCGAGTTCG AGCGCAGCGA CGGCCACGTC 240

GAGCACCGCC GCAAGCTGCA CCCGTACCGG GTGAAGGGCA GCATGGCGAC GATGAAGGTC 300

GTGCACGACC TCGGCCGCGC GGCGGCGGAG CCGGTCCGGT GA 342

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 312 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTGACGGCCG GGCAGGTGCG GGTCCTGGTC CGCTACCAGG CTCCGGGCGA CGACCCCGAG 60

GCCGTCGTCC AGGCGTACAA GCTGGTCTGC GAGGAACTGC GCGGGACGCC CGGCCTGCTC 120

GGCAGCGAGC TGCTGGCGTC CACGCTCGAC GAGGGACGGT TCGCGGTGCT GAGCCTGTGG 180

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AGCGACGCCG CGCGGTTCCA GGAATGGGAG CAGGGCCCGG CGCACAAGGG CCAGACGTCC 240

GGCCTGCGCC CGTTCCGGGA CACCTCCTCG GGGCGCGGCT TCGATTTCTA CGAAGTGGTG 300

CACGCCCTGT AA 312

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1236 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGCCCTCCT CGAAGGATGC CCCGACCGTC GACCCCCGCC CCGACGTCAC GCCGGCCTTC 60

CCGTTCCGGC CGGACGACCC CTTCCAGCCG CCGTGCGAGC ACGCGCGCCT GCGCGCGTCC 120

GACCCGGTCG CCAAGGTGGT GCTGCCGACC GGCGACCACG CGTGGGTCGT GACGCGCTAC 180

GCCGACGTCC GGTTCGTCAC CTCGGACCGG CGCTTCAGCA AGGAGGCGGT GACCCGGCCG 240

GGCGCGCCGC GCCTGATCCC GATGCAGCGC GGCTCCAAGT CGCTGGTCAT CATGGACCCG 300

CCCGAGCACA CGAGGATGCG CAAGATCGTG TCTCGCGCGT TCACCGCCCG TCGTGTGGAG 360

GGGATGCGCG CGCACGTGCG CGACCTGACG TCGGGGTTCG TGGACGAGAT GGTCGAGCAC 420

GGCCCGCCCG CCGACCTGAT CGCGCACCTG GCGCTGCCGC TGCCGGTCAC CGTCATCTGC 480

GAGATGCTGG GCGTTCCGCC GGAGGATCGG CCGCGCTTCC AGGACTGGAC CGACCGGATG 540

CTCACCATCG GCGCGCCCGC TCTCGCGCAG GCCGACGAGA TCAAGGCCGC GGTCGGGCGG 600

CTGCGCGGCT ACCTCGCCGA GCTGATCGAC GCCAAGACGG CCGCGCCCGC CGACGACCTG 660

CTGTCGTTGC TGAGCCGCGC GCACGCCGAC GACGGCCTCA GCGAGGAGGA ACTGCTCACC 720

TTCGGCATGA CGCTGCTGGC GGCGGGTTAC CACACCACCA CGGCGGCGAT CACGCACTCG 780

GTCTACCACC TGCTGCGCGA GCCGTCGCGG TACGCGCGGC TGCGCGAGGA CCCGTCGGGT 840

ATCCCGGCGG CCGTTGAGGA ACTGCTACGG TACGGGCAGA TCGGCGGCGG CGCGGGCGCG 900

ATCCGCATCG CGGTCGAGGA TGTGGAAGTC GGCGGCACCC TCGTGCGCGC GGGCGAGGCG 960

GTCATCCCGC TTTTCAACGC CGCCAACCGC GATCCGGAGG TGTTCGCCGA TCCCGAGGAA 1020

CTCGACCTCG GCCGTACCGA CAACCCGCAC ATCGCGCTCG GCCACGGCAT CCACTACTGC 1080

CTGGGCGCGC CGCTCGCCCG GCTGGAGCTT CAGGTCGTGC TGGAGACGCT GGTGGAGCGG 1140

ACGCCCGCGC TGCGTCTCGC TATCGACGAT GCCGACATCA CCTGGCGGCC CGGCTTGGCG 1200

TTCGCGCGGC CGGACGCGCT GCCCATCGCC TGGTAG 1236

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 347 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATGGACAGGT TCCTGATCGT CGCCCGCATG TCCCCCTCGT CGGAGAAGGA GGTGGCGCGC 60

CTGTTCGCCG AGTCCGAACG AGGGCACCGA GCTGCCGGAG GTGGCCGGGA CGGTCAGCCG 120

CAGCCTGCTG TCGTTCCACG GCCTGTACTT CCACCTGACG GAGGTGGAGG AGAGCACGGA 180

CAGGACGCTG AACGGCATCC ACGAACACCC CGAGTTCGTC CGGCTGAGCC GCCAGCTGTC 240

CGGTCACGTC CAGGCGTACG AACCCGAAGA CGTGGCGCTC GCCCGCCGAC GCCATGGCCC 300

GCGAGTTCTA CCGGTGGGAG GCGGGGACCG GCGTCGTGCG CCGCTGA 347

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 425 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Ser Arg Pro Gln Gly Gly Gly Pro Arg Arg Val Ala Ile Thr Gly
1 5 10 15

Met Gly Val Val Ala Pro Gly Gly Ser Gly Arg Lys Ala Phe Trp Asn
20 25 30

Leu Leu Thr Asp Gly Arg Thr Ala Thr Arg Lys Ile Ser Leu Phe Asp
35 40 45

Pro Ala Gly Phe Arg Ser Arg Ile Ala Ala Glu Cys Asp Phe Asp Pro
50 55 60

Ala Ala Glu Gly Leu Thr Pro Arg Glu Val Arg Arg Met Asp Arg Ala
65 70 75 80

Ala Gln Leu Ala Val Val Ser Ala Arg Glu Ala Leu Ala Asp Ser Gly
85 90 95

Leu Val Ala Gly Glu Gly Asp Pro Ala Arg Phe Ala Val Ser Leu Gly
100 105 110

Ser Ala Val Gly Cys Thr Met Gly Leu Glu Asp Glu Tyr Val Val Val
115 120 125

Ser Asp Gln Gly Arg Asp Trp Leu Val Asp His Ser Tyr Gly Val Pro
130 135 140

His Leu Tyr Arg His Leu Val Pro Ser Ser Leu Ala Ala Glu Val Ala
145 150 155 160

Trp Ala Gly Gly Ala Glu Gly Pro Val Thr Leu Ile Ser Thr Gly Cys
165 170 175

Thr Ser Gly Leu Asp Ala Val Gly His Gly Ala Arg Val Ile Ala Glu
180 185 190

```
Gly Ser Ala Asp Val Ala Leu Ala Gly Ala Thr Asp Ala Pro Ile Ser
        195                 200                 205

Pro Ile Thr Val Ala Cys Phe Asp Ala Ile Arg Ala Thr Ser Pro Asn
    210                 215                 220

Asn Asp Asp Pro Glu His Ala Ser Arg Pro Phe Asp Arg Glu Arg Asn
225                 230                 235                 240

Gly Phe Val Leu Gly Glu Gly Ala Ala Val Phe Val Leu Glu Glu Leu
                245                 250                 255

Glu His Ala Arg Arg Arg Gly Ala His Val Tyr Cys Glu Val Ala Gly
            260                 265                 270

Tyr Ala Thr Arg Gly Asn Ala Tyr His Met Thr Gly Leu Lys Pro Asp
        275                 280                 285

Gly Arg Glu Met Ala Glu Ala Ile Arg Val Ala Met Asp Ala Ala Arg
    290                 295                 300

Val Ala Pro Ala Asp Leu Asp Tyr Ile Asn Ala His Gly Ser Gly Thr
305                 310                 315                 320

Lys Gln Asn Asp Arg His Glu Thr Ala Ala Phe Lys Arg Ser Leu Gly
                325                 330                 335

Glu Arg Ala Tyr Glu Leu Pro Val Ser Ser Ile Lys Ser Met Val Gly
            340                 345                 350

His Ser Leu Gly Ala Ile Gly Ser Ile Glu Leu Ala Ala Cys Ala Leu
        355                 360                 365

Ala Ile Glu His Gly Val Val Pro Pro Thr Ala Asn Leu His Asn Ala
    370                 375                 380

Asp Pro Glu Cys Asp Leu Asp Tyr Val Pro Leu Val Ala Arg Glu Gly
385                 390                 395                 400

Arg Ile Arg Thr Val Leu Ser Val Gly Ser Gly Phe Gly Gly Phe Gln
                405                 410                 415

Ser Ala Thr Val Leu Arg Glu Ala Ala
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 407 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ser Val Leu Thr Ala Asp Ala Pro Ala Val Thr Gly Ile Gly Val
1 5 10 15

Val Ala Pro Thr Gly Ile Gly Val Glu Glu His Trp Ala Ala Thr Leu
20 25 30

Arg Gly Val Pro Val Ile Gly Pro Leu Thr Arg Phe Asp Ala Ser Arg
35 40 45

Tyr Pro Ser Pro Phe Gly Gly Glu Val Pro Gly Phe Asp Ala Ala Glu
50 55 60

Arg Val Pro Gly Arg Leu Ile Pro Gln Thr Asp His Trp Thr His Leu
65 70 75 80

Ala Leu Ala Ala Thr Asp Leu Ala Leu Ala Asp Ala Gly Val Val Pro
85 90 95

Ala Glu Leu Pro Glu Tyr Glu Met Ala Val Val Thr Ala Ser Ser Ser
100 105 110

Gly Gly Val Glu Phe Gly Gln Arg Glu Ile Gln Ala Leu Trp Arg Asp
115 120 125

Gly Pro Arg His Val Gly Ala Tyr Gln Ser Ile Ala Trp Phe Tyr Ala
130 135 140

Ala Thr Thr Gly Gln Ile Ser Ile Arg His Gly Met Arg Gly Pro Cys
145 150 155 160

Gly Val Val Val Ala Glu Gln Ala Gly Ala Leu Glu Ser Phe Ala Gln
165 170 175

Ala Arg Arg Tyr Leu Ala Asp Gly Ala Arg Val Val Val Ser Gly Gly
180 185 190

Thr Asp Ala Pro Phe Ser Pro Tyr Gly Leu Thr Cys Gln Leu Gly Ser
195 200 205

Gly Arg Leu Ser Thr Gly Ala Asp Pro Ala Arg Ala Tyr Leu Pro Phe
210 215 220

Asp Ala Ala Ala Asn Gly Phe Val Pro Gly Glu Gly Gly Ala Ile Leu
225 230 235 240

Ile Ile Glu Gln Ala Ala Thr Ala Gln Asp Arg Ser Tyr Gly Arg Ile
245 250 255

Ala Gly Tyr Ala Ala Thr Phe Asp Pro Pro Pro Gly Ser Gly Arg Pro
260 265 270

Pro Thr Leu Glu Arg Ala Val Arg Ala Ala Leu Asp Asp Ala Arg Leu
275 280 285

Thr Pro Ala Asp Val Asp Val Val Phe Ala Asp Ala Ala Gly Val Pro
290 295 300

Asp Leu Asp Arg Ala Glu Ala Asp Ala Ile Gly Ala Val Phe Gly Pro
305 310 315 320

Arg Gly Val Pro Val Thr Ala Pro Lys Ser Leu Thr Gly Arg Leu Tyr
325 330 335

Ala Gly Gly Pro Ala Leu Asp Ala Ala Thr Ala Leu Leu Ala Met His
340 345 350

Asp Ser Val Ile Pro Pro Thr Ala Gly Gly Ala Asp Val Pro Pro Gly
355 360 365

Tyr Ala Leu Asp Leu Val Gly Ala Glu Pro Arg Pro Ala Arg Leu Arg
370 375 380

Thr Ala Leu Ile Ile Ala Arg Gly Tyr Gly Gly Phe Asn Ala Ala Leu
385 390 395 400

Val Leu Arg Gly Pro Asn Thr
405

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 87 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Ala Thr Arg Glu Arg Thr Ile Asp Asp Leu Arg Ala Leu Met Arg
1 5 10 15

Ala Ala Val Gly Glu Ala Asp Asp Ile Asp Leu Asp Gly Asp Ile Leu
20 25 30

Asp Ser Thr Phe Thr Glu Leu Glu Tyr Asp Ser Leu Ala Val Leu Glu
35 40 45

Leu Ala Ala Arg Ile Glu Thr Gln Trp Gly Val Leu Ile Pro Glu Asp
50 55 60

Asp Ala Ser Gly Leu Glu Thr Pro Arg Met Phe Leu Asp Tyr Val Asn
65 70 75 80

Gly Arg Ala Val Ala Glu Arg
85

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 153 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Thr Gln Trp Arg Thr Asp Ser Val Ile Val Ile Asp Ala Pro Leu
1 5 10 15

Asp Val Val Trp Asp Met Thr Asn Asp Val Ala Ser Trp Pro Glu Leu
20 25 30

---

Phe Asp Glu Tyr Ala Ser Ala Glu Ile Leu Glu Arg Asp Gly Asp Thr
35 40 45

Val Arg Phe Arg Leu Thr Met His Pro Asp Ala Asp Gly Asn Ala Trp
50 55 60

Ser Trp Val Ser Glu Arg Thr Pro Asp Arg Ala Ala Leu Thr Val Asn
65 70 75 80

Ala His Arg Val Glu Thr Gly Trp Phe Glu His Met Asn Leu Arg Trp
85 90 95

Asp Tyr Arg Glu Val Pro Gly Gly Val Glu Met Arg Trp Arg Gln Asp
100 105 110

Phe Ala Met Lys Glu Ala Ser Pro Val Ser Leu Ala Ala Met Thr Glu
115 120 125

Arg Ile Gln Ser Asn Ser Pro Val Gln Met Lys Leu Ile Lys Asp Lys
130 135 140

Val Glu Arg Ala Ala Arg Gly Ala Arg
145 150

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 153 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Ile Glu Phe Leu Leu Pro Val Ala Leu Leu Gly Asn Gly Leu Cys
1 5 10 15

Ala Gly Val Leu Thr Gly Ser Val Leu Gly Val Val Pro Tyr Tyr Arg
20 25 30

Thr Leu Pro Glu Asp Arg Tyr Ile Ala Ala His Ala Phe Ala Val Gly
35 40 45

---

Arg Tyr Asp Pro Phe Gln Pro Val Cys Leu Leu Val Thr Val Ala Ala
50 55 60

Asp Ala Val Ala Ala Ala Val Ala Pro Thr Ala Ala Ala Arg Val Leu
65 70 75 80

Cys Ala Leu Ala Ala Val Leu Ala Leu Ala Val Val Ala Ile Ser Leu
85 90 95

Thr Arg Asn Val Pro Met Asn Arg Arg Ile Lys Arg Leu Asp Pro Ala
100 105 110

Ala Pro Pro Ala Gly Phe Ser Ala Pro Ala Phe Leu Arg Arg Trp Ala
115 120 125

Gly Trp Asn Ala Ala Arg Thr Gly Leu Thr Leu Ala Ala Leu Leu Ser
130 135 140

Asn Thr Ala Ala Leu Gly Val Leu Leu
145 150

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 341 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Thr Glu Pro Glu Gly Pro His Ala Ala Ser Leu Arg Leu Gln Ser
1 5 10 15

Leu Leu Asp Gly Met Arg Val Ala Lys Val Val Gln Val Leu Ala Glu
20 25 30

Leu Gln Val Ala Asp Ala Val Ala Asp Gly Pro Cys Lys Pro Ala Glu
35 40 45

Ile Ala Ala Asp Val Gly Ala Asp Pro Asp Ala Leu Tyr Arg Val Leu

```
    50                  55                  60

Arg Cys Ala Ala Ser Phe Gly Val Phe Thr Glu Asp Glu Asp Gly Arg
65                  70                  75                  80

Phe Gly Leu Thr Pro Met Ala Ala Leu Leu Arg Thr Gly Thr Asp Asp
                85                  90                  95

Ser His Arg Asp Leu Phe Met Met Ala Ala Gly Asp Leu Trp Trp Arg
            100                 105                 110

Pro Tyr Gly Glu Leu Leu Glu Thr Val Arg Thr Gly Arg Pro Ala Ala
        115                 120                 125

Glu Leu Ala Phe Gly Met Pro Phe Tyr Asp Tyr Leu Gly Thr Asp Pro
    130                 135                 140

Ala Ala Ala Gly Leu Phe Asp Arg Ala Met Thr Gln Val Ser Lys Gly
145                 150                 155                 160

Gln Ala Lys Ala Ile Leu Gly Arg Cys Ser Phe Glu Arg Tyr Ala Arg
                165                 170                 175

Ile Ala Asp Val Gly Gly Gly His Gly Tyr Phe Leu Ala Gln Val Leu
            180                 185                 190

Arg Ser Ser Pro Arg Thr Glu Gly Val Leu Leu Asp Leu Pro His Val
        195                 200                 205

Val Ala Gly Ala Pro Ala Val Leu Glu Lys His Glu Val Ala Asp Arg
    210                 215                 220

Val Gln Val Val Pro Gly Ser Phe Phe Asp Ala Leu Pro Thr Gly Cys
225                 230                 235                 240

Asp Ala Tyr Leu Leu Lys Ala Ile Leu Ile Asn Trp Pro Asp Ala Asp
                245                 250                 255

Ala Glu Arg Ile Leu His Arg Val Arg Glu Ala Ile Gly Thr Asp Arg
            260                 265                 270

Asp Ala Arg Leu Leu Val Val Glu Pro Val Val Pro Pro Gly Asp Val
        275                 280                 285

Arg Asp Tyr Ser Lys Ala Thr Asp Ile Asp Met Leu Ala Ile Ile Gly
    290                 295                 300

Gly Arg Gln Arg Thr Val Ala Glu Trp Arg Arg Leu Leu Arg Ala Gly
305                 310                 315                 320

Gly Phe Glu Leu Val Gly Glu Pro Thr Pro Gly Arg Arg Glu Val Met
```

325 330 335

Glu Cys Arg Pro Ile
340

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 246 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Thr Asp Thr Ser Phe Ala Gly Lys Asn Ala Leu Ile Thr Gly Gly
1 5 10 15

Thr Arg Gly Ile Gly Arg Ala Val Ala Leu Gly Leu Ala Arg Ala Gly
20 25 30

Ala Asn Val Thr Val Cys Tyr Arg Ser Asp Ala Glu Ser Ala Ala Ala
35 40 45

Met Glu Ala Glu Leu Ala Ala Thr Asp Gly Lys His His Val Leu Gln
50 55 60

Ala Asp Ile Gly Asn Ala Gly Asp Val Arg Arg Leu Leu Asp Glu Val
65 70 75 80

Ala Ala Arg Met Gly Ser Leu Asp Val Val Val His Asn Ala Gly Leu
85 90 95

Ile Ser His Val Pro Phe Ala Asp Leu Glu Pro Glu Glu Trp His Arg
100 105 110

Ile Val Asp Ser Asn Leu Thr Gly Met Tyr Leu Val Val Arg Ala Ala
115 120 125

Leu Pro Leu Leu Ser Glu Gly Gly Ala Val Val Gly Val Gly Ser Lys
130 135 140

Val Ala Leu Val Gly Ile Ser Gln Arg Thr His Tyr Thr Ala Ala Lys
145 150 155 160

Ala Gly Leu Ile Gly Phe Val Arg Ser Leu Ser Lys Glu Leu Gly Pro
165 170 175

Leu Gly Ile Arg Val Asn Leu Val Ala Pro Gly Ile Thr Glu Thr Asp
180 185 190

Gln Ala Ala His Leu Pro Pro Val Gln Arg Glu Arg Tyr Gln Ser Met
195 200 205

Thr Ala Leu Lys Arg Leu Gly Gln Ala Asp Glu Val Ala Asp Val Val
210 215 220

Leu Phe Leu Ala Gly Pro Gly Ala Arg Tyr Val Thr Gly Glu Thr Val
225 230 235 240

Asn Val Asp Gly Gly Met
245

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Val Thr Met Ala Asp Ser Gly Pro Val Phe Arg Val Met Leu Arg Met
1 5 10 15

Glu Ile Val Pro Gly Arg Glu Ala Glu Phe Glu Arg Val Trp Tyr Ser
20 25 30

Val Gly Asp Thr Val Ser Gly Asn Pro Ala Asn Leu Gly Gln Cys Val
35 40 45

Leu Arg Ser Asp Asp Glu Glu Ser Val Tyr Tyr Ile Met Ser Asp Trp
50 55 60

Ile Asp Glu Ala Arg Phe Arg Glu Phe Glu Arg Ser Asp Gly His Val
65 70 75 80

Glu His Arg Arg Lys Leu His Pro Tyr Arg Val Lys Gly Ser Met Ala
85 90 95

Thr Met Lys Val Val His Asp Leu Gly Arg Ala Ala Ala Glu Pro Val
100 105 110

Arg

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 103 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Val Thr Ala Gly Gln Val Arg Val Leu Val Arg Tyr Gln Ala Pro Gly
1 5 10 15

Asp Asp Pro Glu Ala Val Val Gln Ala Tyr Lys Leu Val Cys Glu Glu
20 25 30

Leu Arg Gly Thr Pro Gly Leu Leu Gly Ser Glu Leu Leu Ala Ser Thr
35 40 45

Leu Asp Glu Gly Arg Phe Ala Val Leu Ser Leu Trp Ser Asp Ala Ala
50 55 60

Arg Phe Gln Glu Trp Glu Gln Gly Pro Ala His Lys Gly Gln Thr Ser
65 70 75 80

Gly Leu Arg Pro Phe Arg Asp Thr Ser Ser Gly Arg Gly Phe Asp Phe
85 90 95

Tyr Glu Val Val His Ala Leu

100

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 411 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Pro Ser Ser Lys Asp Ala Pro Thr Val Asp Pro Arg Pro Asp Val
1 5 10 15

Thr Pro Ala Phe Pro Phe Arg Pro Asp Asp Pro Phe Gln Pro Pro Cys
20 25 30

Glu His Ala Arg Leu Arg Ala Ser Asp Pro Val Ala Lys Val Val Leu
35 40 45

Pro Thr Gly Asp His Ala Trp Val Val Thr Arg Tyr Ala Asp Val Arg
50 55 60

Phe Val Thr Ser Asp Arg Arg Phe Ser Lys Glu Ala Val Thr Arg Pro
65 70 75 80

Gly Ala Pro Arg Leu Ile Pro Met Gln Arg Gly Ser Lys Ser Leu Val
85 90 95

Ile Met Asp Pro Pro Glu His Thr Arg Met Arg Lys Ile Val Ser Arg
100 105 110

Ala Phe Thr Ala Arg Arg Val Glu Gly Met Arg Ala His Val Arg Asp
115 120 125

Leu Thr Ser Gly Phe Val Asp Glu Met Val Glu His Gly Pro Pro Ala
130 135 140

Asp Leu Ile Ala His Leu Ala Leu Pro Leu Pro Val Thr Val Ile Cys
145 150 155 160

Glu Met Leu Gly Val Pro Pro Glu Asp Arg Pro Arg Phe Gln Asp Trp
165 170 175

Thr Asp Arg Met Leu Thr Ile Gly Ala Pro Ala Leu Ala Gln Ala Asp
180 185 190

Glu Ile Lys Ala Ala Val Gly Arg Leu Arg Gly Tyr Leu Ala Glu Leu
195 200 205

Ile Asp Ala Lys Thr Ala Ala Pro Ala Asp Asp Leu Leu Ser Leu Leu
210 215 220

Ser Arg Ala His Ala Asp Asp Gly Leu Ser Glu Glu Glu Leu Leu Thr
225 230 235 240

Phe Gly Met Thr Leu Leu Ala Ala Gly Tyr His Thr Thr Thr Ala Ala
245 250 255

Ile Thr His Ser Val Tyr His Leu Leu Arg Glu Pro Ser Arg Tyr Ala
260 265 270

Arg Leu Arg Glu Asp Pro Ser Gly Ile Pro Ala Ala Val Glu Glu Leu
275 280 285

Leu Arg Tyr Gly Gln Ile Gly Gly Gly Ala Gly Ala Ile Arg Ile Ala
290 295 300

Val Glu Asp Val Glu Val Gly Gly Thr Leu Val Arg Ala Gly Glu Ala
305 310 315 320

Val Ile Pro Leu Phe Asn Ala Ala Asn Arg Asp Pro Glu Val Phe Ala
325 330 335

Asp Pro Glu Glu Leu Asp Leu Gly Arg Thr Asp Asn Pro His Ile Ala
340 345 350

Leu Gly His Gly Ile His Tyr Cys Leu Gly Ala Pro Leu Ala Arg Leu
355 360 365

Glu Leu Gln Val Val Leu Glu Thr Leu Val Glu Arg Thr Pro Ala Leu
370 375 380

Arg Leu Ala Ile Asp Asp Ala Asp Ile Thr Trp Arg Pro Gly Leu Ala
385 390 395 400

Phe Ala Arg Pro Asp Ala Leu Pro Ile Ala Trp
405 410

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Asp Arg Phe Leu Ile Val Ala Arg Met Ser Pro Ser Ser Glu Lys
1 5 10 15

Glu Val Ala Arg Leu Phe Ala Glu Ser Asp Glu Gly Thr Glu Leu Pro
20 25 30

Glu Val Ala Gly Thr Val Ser Arg Ser Leu Leu Ser Phe His Gly Leu
35 40 45

Tyr Phe His Leu Thr Glu Val Glu Glu Ser Thr Asp Arg Thr Leu Asn
50 55 60

Gly Ile His Glu His Pro Glu Phe Val Arg Leu Ser Arg Gln Leu Ser
65 70 75 80

Gly His Val Gln Ala Tyr Asp Pro Lys Thr Trp Arg Ser Pro Ala Asp
85 90 95

Ala Met Ala Arg Glu Phe Tyr Arg Trp Glu Ala Gly Thr Gly Val Val
100 105 110

Arg Arg

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "probe"

---

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGCGCGGAGG GCCCGGTCAC GATGGTCTCC ACCGGCTGCA CCTCGGGCCT GGAC 54

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "probe"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CCCGTCAGCT CCATCAAGTC CATGGTCGGC CACTCGCTCG GCGCGATCGG CTCC 54

**WE CLAIM:**

1. A substantially pure nucleic acid comprising a nucleic acid encoding a polypeptide sharing at least about 75% amino acid identity with an *Actinomadura* polyketide synthase.

2. The nucleic acid of claim 1, encoding a polypeptide sharing at least about 80% amino acid identity with an *Actinomadura* polyketide synthase.

3. The nucleic acid of claim 2, encoding a polypeptide sharing at least about 90% amino acid identity with an *Actinomadura* polyketide synthase.

4. The substantially pure nucleic acid of claim 1, comprising a nucleic acid selected from the group consisting of SEQ ID NO:1-12.

5. A transformed eukaryotic or prokaryotic cell comprising the nucleic acid of claim 1.

6. A vector capable of reproducing in a eukaryotic or prokaryotic cell comprising the nucleic acid of claim 1.

7. A substantially pure nucleic acid comprising a nucleic acid that hybridizes to the nucleic acid of claim 1 under stringent conditions.

8. A substantially pure nucleic acid comprising a nucleic acid encoding a polypeptide sharing at least about 75% amino acid identity with a polyketide synthase for biosynthesis of a benzo(*a*)naphthacenequinone.

9. The substantially pure nucleic acid of claim 8, encoding a polypeptide sharing at least about 80% amino acid identity with a polyketide synthase for biosynthesis of a benzo(*a*)naphthacenequinone.

10. The nucleic acid of claim 9, encoding a polypeptide sharing at least about 90% amino acid identity with a polyketide synthase for biosynthesis of a benzo(*a*)naphthacenequinone.

11. The nucleic acid of claim 10, wherein the polyketide synthase is an *Actinomadura* polyketide synthase.

12. The nucleic acid of claim 11, wherein the polyketide synthase is an *Actinomadura* polyketide synthase.

13. The nucleic acid of claim 12, wherein the polyketide synthase is an *Actinomadura* polyketide synthase.

14. The nucleic acid of claim 8, wherein the benzo(*a*)naphthacenequinone is a dihydrobenzo(*a*)naphthacenequinone aglycon.

15. The nucleic acid of claim 9, wherein the benzo(*a*)naphthacenequinone is a dihydrobenzo(*a*)naphthacenequinone aglycon.

16. The nucleic acid of claim 10, wherein the benzo(*a*)naphthacenequinone is a dihydrobenzo(*a*)naphthacenequinone aglycon.

17. The nucleic acid of claim 14, wherein the dihydrobenzo(*a*)naphthacenequinone aglycon is pradimicin.

18. The nucleic acid of claim 15, wherein the dihydrobenzo(*a*)naphthacenequinone aglycon is pradimicin.

19. The nucleic acid of claim 16, wherein the dihydrobenzo(*a*)naphthacenequinone aglycon is pradimicin.

20. A substantially pure polypeptide comprising an amino acid sequence sharing at least about 75% amino acid identity with an *Actinomadura* polyketide synthase.

21. The polypeptide of claim 20, comprising an amino acid sequence sharing at least about 80% amino acid identity with an *Actinomadura* polyketide synthase.

22. The polypeptide of claim 21, comprising an amino acid sequence sharing at least about 90% amino acid identity with an *Actinomadura* polyketide synthase.

23. The polypeptide of claim 22, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

24. A method of preparing pradimicin or an analog thereof comprising:

(a) transforming a eukaryotic or prokaryotic cell with an expression vector for expressing intracellularly or extracellularly a nucleic acid comprising a nucleic acid encoding a polypeptide sharing at least about 70% amino acid identity with an *Actinomadura* polyketide synthase;

(b) growing the transformed cell in culture; and

(c) isolating the pradimicin or analog thereof from the transformed cell or the culture medium.

25. The method of claim 24, wherein the polypeptide shares at least about 80% amino acid identity with an *Actinomadura* polyketide synthase.

26. The method of claim 25, wherein the polypeptide shares at least about 90% amino acid identity with an *Actinomadura* polyketide synthase.

27. The method of claim 24, wherein the nucleic acid comprises SEQ ID NO:1.

Pradimicin A; R1=H, R2=H
Pradimicin S; R1=$CH_2OH$, R2=$HO_3S$

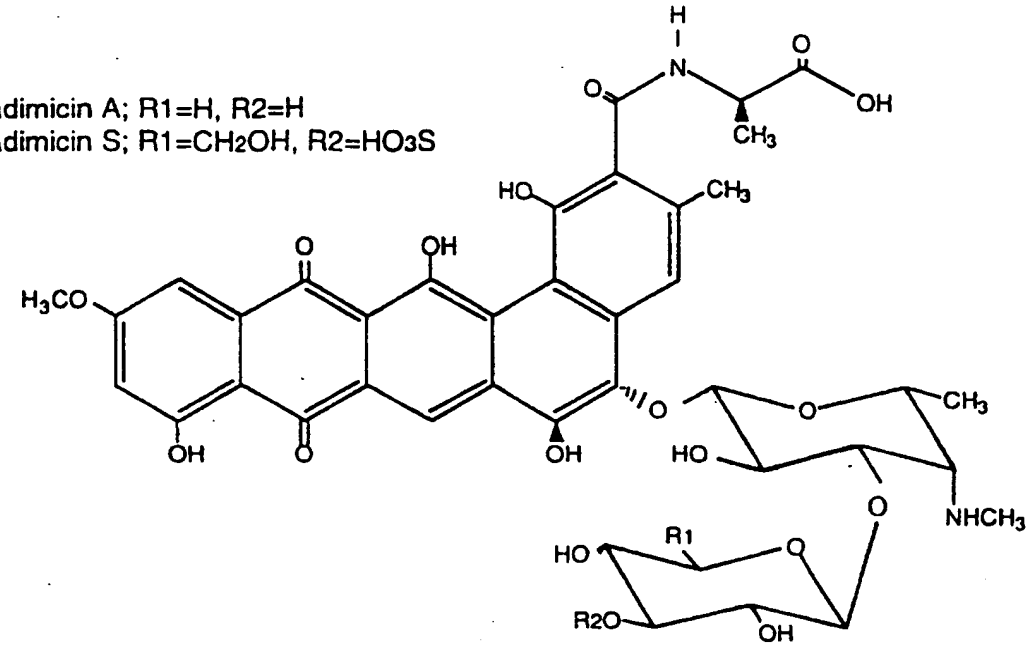


FIGURE 1

## β - Keto synthase

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Granatidin | G | A | E | G | P | V | T | M | V | S | D | G | C | T | S | G | L | D |
| Tetracenomycin | G | A | E | G | P | V | T | V | V | S | T | G | C | T | S | G | L | D |
| Actinorhodin | G | A | E | G | P | V | T | M | V | S | T | G | C | T | S | G | L | D |
| CONSENSUS | G | A | E | G | P | V | T | M | V | S | T | G | C | T | S | G | L | D |

Probe 1 (54 mer) 5'-GGCGCGGAGGGCCCGGTCACGATGGTCTCCACCGGCTGCACCTCGGGCCTGGAC-3'

## Acyl transferase

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Granatidin | P | V | S | S | I | K | S | M | G | G | H | S | L | G | A | I | G | S |
| Tetracenomycin | P | V | S | S | I | K | S | M | I | G | H | S | L | G | A | I | G | S |
| Actinorhodin | P | V | S | S | I | K | S | M | V | G | H | S | L | G | A | I | G | S |
| CONSENSUS | P | V | S | S | I | K | S | M | ( ) | G | H | S | L | G | A | I | G | S |

Probe 2 (54 mer) 5'-CCCGTCAGCTCCATCAAGTCCATGGTCGGCCACTCGCTCGGCGCGATCGGCTCC-3'

FIGURE 2

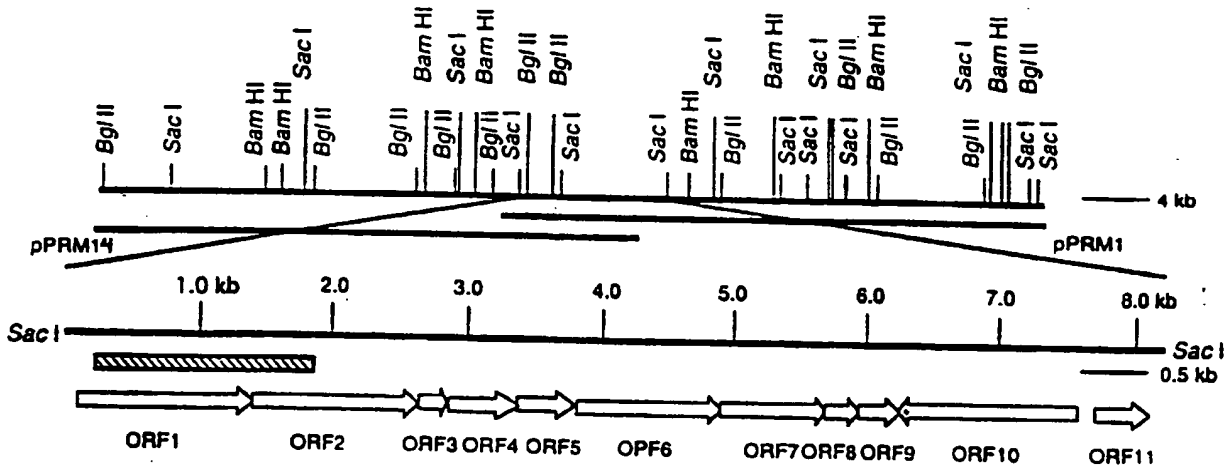
Bgl II
Sac I
Bam HI
Bam HI
Sac I
Bgl II
Bgl II
Bam HI
Sac I
Bam HI
Bgl II
Bgl II
Bgl II
Sac I
Sac I
Sac I
Bam HI
Sac I
Bgl II
Sac I
Sac I
Bam HI
Sac I
Bgl II
Bam HI
Bgl II
Sac I
Bam HI
Bgl II
Sac I
Sac I
4 kb
pPRM14
pPRM1
1.0 kb
2.0
3.0
4.0
5.0
6.0
7.0
8.0 kb
Sac I
Sac I
0.5 kb
ORF1
ORF2
ORF3 ORF4 ORF5
OPF6
ORF7 ORF8 ORF9
ORF10
ORF11

FIGURE 3

```
    1                                                                60
A   MSRPQGGGPRRVAITGMGVVAPGGSGRKAFWNLLTDGRTATRKISLFDPAGFRSRIAAEC
            .**.***.** ****.*  ***.***.******.*****.* .*****.*
B      MTRHAEKRVVITGIGVRAPGGAGTAAFWDLLTAGRTATRTISLFDAAPYRSRIAGEI
       1                                                         57
    DFDPAAEGLTPREVRRMDRAAQLAVVSAREALADSGLVAGEGDPARFAVSLGSAVGCTMG
    **** .***.**...  ***.*****.***** **** ... .*.*..**.*.***** *
    DFDPIGEGLSPRQASTYDRATQLAVVCAREALKDSGLDPAAVNPERIGVSIGTAVGCTTG

    LEDEYVVVSDQGRDWLVDHSYGVPHLYRHLVPSSLAAEVAWAGGAEGPVTLISTGCTSGL
    *. **. **. *. *****. .* .*. ..**.*.  ****..*******..********
    LDREYARVSEGGSRWLVDHTLAVEQLFDYFVPTSICREVAWEAGAEGPVTVVSTGCTSGL

    DAVGHGARVIAEGSADVALAGATDAPISPITVACFDAIRATSPNNDDPEHASRPFDRERN
    ****.*. .* .*.***.. **********.***.*****.********.*.
    DAVGYGTELIRDGRADVVVCGATDAPISPITVACFDAIKATSANNDDPAHASRPFDRNRD

    GFVLGEGAAVFVLEELEHARRRGAHVYCEVAGYATRGNAYHMTGLKPDGREMAEAIRVAM
    *******.********. *******.* ** *.***.**.**************** .*.
    GFVLGEGSAVFVLEELSAARRRGAHAYAEVRGFATRSNAFHMTGLKPDGREMAEAITAAL

    DAARVAPADLDYINAHGSGTKQNDRHETAAFKRSLGERAYELPVSSIKSMVGHSLGAIGS
    *.**  . .**.*********.**************.***. .********.*********
    DQARRTGDDLHYINAHGSGTRQNDRHETAAFKRSLGQRAYDVPVSSIKSMIGHSLGAIGS

    IELAACALAIEHGVVPPTANLHNADPECDLDYVPLVAREGRIRTVLSVGSGFGGFQSATV
    .**********.*****  ...********** **** *. **************.*
    LELAACALAIEHGVIPPTANYEEPDPECDLDYVPNVAREQRVDTVLSVGSGFGGFQSAAV
      425
    LREAA
    *
    LARPK
      422
```

FIGURE 4

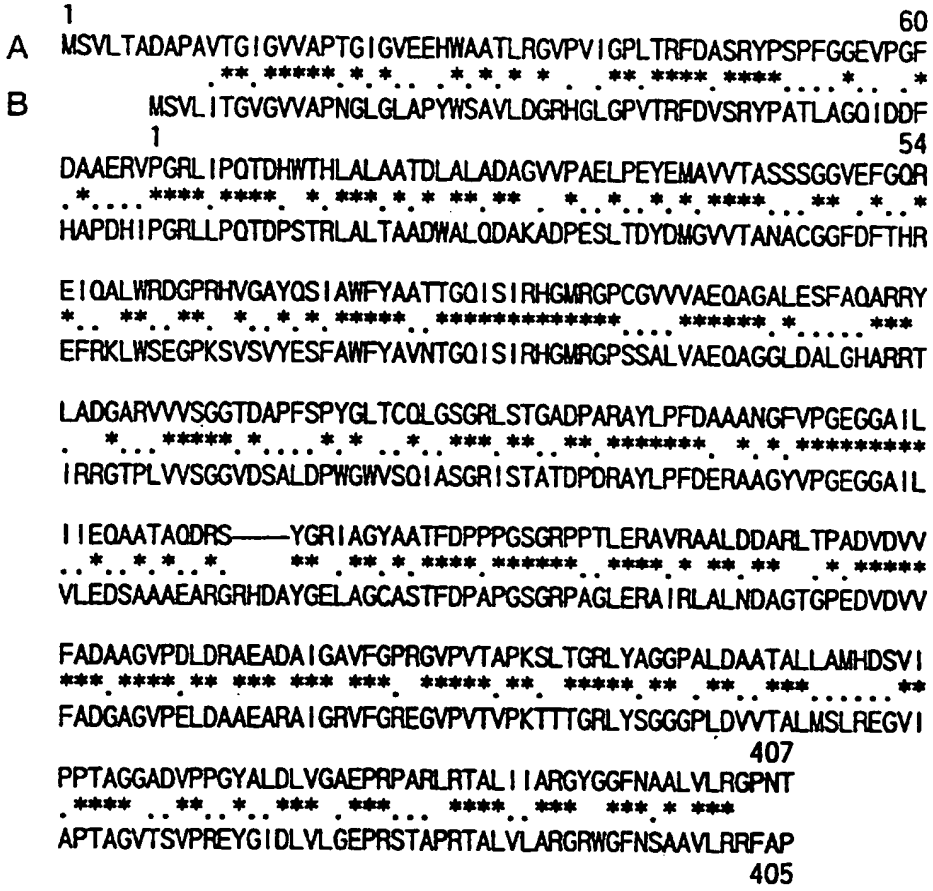
1
60
A MSVLTADAPAVTGIGVVAPTGIGVEEHWAATLRGVPVIGPLTRFDASRYPSPFGGEVPGF
B MSVLITGVGVVAPNGLGLAPYWSAVLDGRHGLGPVTRFDVSRYPATLAGQIDDF
1
54
DAAERVPGRLIPQTDHWTHLALAATDLALADAGVVPAELPEYEMAVVTASSSGGVEFGQR
HAPDHIPGRLLPQTDPSTRLALTAADWALQDAKADPESLTDYDMGVVTANACGGFDFTHR
EIQALWRDGPRHVGAYQSIAWFYAATTGQISIRHGMRGPCGVVVAEQAGALESFAQARRY
EFRKLWSEGPKSVSVYESFAWFYAVNTGQISIRHGMRGPSSALVAEQAGGLDALGHARRT
LADGARVVVSGGTDAPFSPYGLTCQLGSGRLSTGADPARAYLPFDAAANGFVPGEGGAIL
IRRGTPLVVSGGVDSALDPWGWVSQIASGRISTATDPDRAYLPFDERAAGYVPGEGGAIL
IIEQAATAQDRS——YGRIAGYAATFDPPPGSGRPPTLERAVRAALDDARLTPADVDVV
VLEDSAAAEARGRHDAYGELAGCASTFDPAPGSGRPAGLERAIRLALNDAGTGPEDVDVV
FADAAGVPDLDRAEADAIGAVFGPRGVPVTAPKSLTGRLYAGGPALDAATALLAMHDSVI
FADGAGVPELDAAEARAIGRVFGREGVPVTVPKTTTGRLYSGGGPLDVVTALMSLREGVI
407
PPTAGGADVPPGYALDLVGAEPRPARLRTALIIARGYGGFNAALVLRGPNT
APTAGVTSVPREYGIDLVLGEPRSTAPRTALVLARGRWGFNSAAVLRRFAP
405

FIGURE 5

# INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/US 96/14791

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/52 C12N1/21 C12N9/00 C12P21/00 //(C12N1/21, C12R1:03)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | MOL. GEN. GENET., vol. 251, 1996, pages 113-120, XP000652375 K. YLIHONKO ET AL.: "A gene cluster involved in nogalamycin biosynthesis from Streptomyces nogalater: sequence analysis and complementation of early-block mutations in the anthracycline pathway" see the whole document. --- -/-- | 1,6-8, 20,24 |

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 10 July 1997 | 04.08.97 |

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| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. ( + 31-70) 340-2040, Tx. 31 651 epo nl, Fax: ( + 31-70) 340-3016 | Yeats, S |

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# INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 96/14791

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | MOL. GEN. GENET., vol. 240, 1993, pages 146-150, XP000654921 C. LE GOUILL ET AL.: "Saccharopolyspora hirsuta 367 encodes clustered genes similar to ketoacyl synthase, ketoacyl reductase, acyl carrier protein, and biotin carboxyl carrier protein" see the whole document. --- | 1,6-8, 20,24 |
| Y | J. BIOL. CHEM., vol. 267, 1992, pages 19278-19290, XP000652285 M.A. FERNANDEZ-MORENO ET AL.: "Nucleotide sequence and deduced functions of a set of cotranscribed genes of Streptomyces coelicolor A3(2) including the polyketide synthase for the antibiotic actinorhodin" cited in the application see the whole document, especially the abstract and Figure 4. --- | 1-27 |
| Y | J. ANTIBIOTICS, vol. 48, 1995, pages 162-168, XP000654920 K. SAITOH ET AL.: "Pradimicin S, a new pradimicin analog. III. Application of the Frit-FAB LC/MS technique to the elucidation of the pradimicin S biosynthetic pathway" see the whole document. ----- | 1-27 |

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